

ESE GENES AND PROTEINSField of the Invention

This invention relates to novel mammalian proteins encoded by the Ese1 and Ese2 genes which are involved in endocytosis, vesicular trafficking and regulation of the actin cytoskeleton.

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Background of the Invention

Endocytosis via clathrin-coated pits is a multistep process (1, 2). Clathrin heavy and light chains are brought to the plasma membrane through association with a heterotetrameric complex known as clathrin adaptor complex 2, or AP-2. At coated pits, the membrane is bent through the assembly of clathrin triskelion into a caged lattice. The GTPase Dynamin is also recruited to the neck of coated pits where it assembles into a collar for vesicle fission (4, 5). Recruitment of Dynamin to coated pits is believed to require the Amphiphysin I/II heterodimer (6), as these proteins bind Dynamin *in vivo* and amphiphysin proteins are required for endocytosis in yeast (7). In addition, ectopic expression of either Amphiphysin I or II by themselves (6), or the isolated SH3 domain of Amphiphysin I blocks endocytosis (8, 9). Recent data has revealed that the Rab5 small GTPase is required for sequestration of ligands such as transferrin and Epidermal Growth Factor into coated pits *in vitro* (10). The mechanism by which these components interact to regulate coated pit assembly, cargo sequestration, followed by vesicle fission is not yet understood.

From biochemical, cell biological and genetic analysis it is clear that additional components such as kinases, phosphatases, ubiquitin conjugating enzymes as well as lipid modifying enzymes are required for clathrin-coat and vesicle formation (1, 11, 12, 13, 14, 15, 16, 17, 18, 19). Indeed there is also strong evidence for a requirement of the actin cytoskeleton in endocytosis and several proteins which may facilitate this connection (1, 7, 20, 21, 22, 23, 24, 25).

The Eps15 protein was discovered in a search for substrates of the Epidermal Growth Factor Receptor (26). In 1995, Benmerah et. al. reported that Eps15 is constitutively associated with  $\alpha$ -adaptin of the AP2 complex (27). The Eps15 protein has also been localized to the neck of clathrin-coated pits by immunoelectron microscopy (28, 29). Recently, two groups have used dominant inhibitory mutants of Eps15, or antibodies against Eps15 (or the related protein Eps15R), to demonstrate that Eps15 proteins are required for endocytosis via clathrin-coated pits (30, 31). Eps15 contains three large structural domains (26, 32). The N-terminal third contains three copies of an EH domain (for Eps15 Homology domain) (32, 33). The central region of Eps15 forms an extended coiled-coil, which is followed by a complex C-

terminus containing SH3-binding motifs (34), a large number of DPF repeats (Aspartic acid-Proline-Phenylalanine), and  $\alpha$ -adaptin binding sequences (31, 35, 36). The full length Eps15R protein has a similar overall organization (34). Both Eps15 and Eps15R can be alternatively spliced to produce numerous smaller proteins(37).

5 A protein with similar overall organization has been identified in *Saccharomyces cerevisiae* named Pan1p. Genetic analysis of *PAN1* has revealed that this gene is required for endocytosis and for organization of the actin cytoskeleton (23, 38). Like Eps15 and Eps15R in mammals, the Pan1p protein has N-terminal EH domains followed by a central coiled-coil domain and C-terminal proline-rich  
10 sequences. A second EH domain containing protein, End3p, has also been described in *S. cerevisiae* which is required for endocytosis and regulation of the actin cytoskeleton (21, 39). Co-immunoprecipitation studies have shown that Pan1p and End3p form a complex *in vivo* (40). Indeed, overexpression of End3p can suppress the phenotype of *pan1-4* hypomorphic mutants, and Pan1p is mislocalized in *end3*  
15 mutants indicating that these proteins function together (40). Additional studies have revealed that the EH domains of Pan1p bind to yeast homologues of mammalian clathrin-binding proteins, AP180 and CALM (yAP180A and yAP180B), through NPF motifs (Asparagine-Proline-Phenylalanine) in the yAP180 C-termini(25). These data have led to a proposal that the Pan1p:End3p complex functions as a multivalent  
20 adaptor to coordinate protein-protein interactions during endocytosis (25, 40). At least two additional proteins are predicted to bind to the Pan1p:End3p complex *in vivo*, as strong genetic interactions have been detected between *PAN1* and *SJL1*(25), and between *PAN1* and *RSP5* (41). *SJL1* encodes a phosphatidylinositol polyphosphate-5-phosphatase protein which is related to mammalian synaptojanin  
25 (42) and has a C-terminal NPF motif predicted to bind to EH domains in Pan1p (or End3p) (25, 43). *RSP5* encodes an E3 ubiquitin-protein ligase which may bind to the C-terminal polyproline sequences in Pan1p through one of its three WW domains (25).

30 Numerous SH3 domain containing proteins have been implicated in the regulation of endocytosis (44). These include Amphiphysin I(45) and II (6, 46, 47, 48), Rsv161/Rsv167(7), Actin Binding Protein-1(49), Endophilin/SH3P4/8/13 (50, 51) and Grb2 (52). Kay and coworkers have reported the isolation of several novel SH3 encoding cDNAs (53).

35 The present inventors have identified novel mammalian proteins containing both EH and SH3 domains, which have been named Ese1 and Ese2. Sequence and functional analysis of the full length proteins have implicated these proteins in receptor mediated endocytosis via clathrin coated pits and therefore the proteins have

been named Ese1 and Ese2 respectively (Ese: for EH-domain and SH3 domain regulator of Endocytosis). Also identified are several mammalian alternative transcript proteins two of which are named Ese1L and Ese2L.

5    **Summary of the Invention**

In accordance with one series of embodiments, this invention provides isolated nucleic acids corresponding to or relating to the nucleic acid sequences disclosed herein which encode the mammalian Ese1 and Ese2 proteins.

10    The invention more specifically provides isolated nucleic acids corresponding to or relating to the nucleic acid sequences disclosed herein which encode the mouse Ese1 and Ese2 proteins.

15    One of ordinary skill in the art is now able to identify and isolate mammalian Ese protein genes or cDNAs which are allelic variants of the disclosed sequences or are homologues thereof, in other species, including humans, using standard hybridisation screening and PCR techniques. The mammalian polynucleotide may be in the form of DNA, genomic DNA, cDNA, mRNA and various fragments and portions of the gene sequence encoding Ese proteins.

20    Homologues of the mammalian Ese protein genes are generally those sequences which share at least 80% sequence identity, preferably at least 90% sequence identity to the mammalian Ese gene sequence.

In a further embodiment, the invention provides cDNA sequences encoding murine Ese1 and Ese2 proteins comprising the nucleotide sequences of Sequence ID NOS: 1, 2, 4 and 5.

25    In a further embodiment, the invention provides cDNA sequences encoding murine Ese1L and Ese2L proteins comprising the nucleotide sequences of Sequence ID NOS: 22, 23, 25 and 26.

30    Also provided are portions of the Ese gene sequences useful as probes or PCR primers or for encoding fragments, functional domains or antigenic determinants of Ese proteins. The probes can be routinely used to screen and identify homologues of the Ese gene or portions thereof while primers are useful in PCR assays for the amplification of desired portions of the selected Ese gene.

35    The invention also provides portions of the disclosed nucleic acid sequences comprising about 10 consecutive nucleotides to nearly the complete disclosed nucleic acid sequences. The invention provides isolated nucleic acid sequences comprising sequences corresponding to at least 10, preferably 15 and more preferably at least 20 consecutive nucleotides of the Ese genes as disclosed or enabled herein or their complements.

In addition, the isolated nucleic acids of the invention include any of the above described nucleotide sequences included in a vector. Expression vectors comprising the nucleotide sequences are provided along with suitable host cells transfected with such expression vectors.

5 In accordance with a further series of embodiments, this invention provides substantially pure mammalian Ese proteins, fragments of these proteins and fusion proteins including these proteins and fragments.

In accordance with a further series of embodiments, this invention provides substantially pure mutant mammalian Ese proteins, fragments of these proteins and  
10 fusion proteins including these mutant proteins and fragments.

In a further embodiment, the invention provides amino acid sequences encoding murine Ese1 and Ese2 proteins comprising the amino acid sequences of Sequence ID NOS: 3 and 6.

In a further embodiment, the invention provides amino acid sequences  
15 encoding murine Ese1L and Ese2L proteins, which are alternative transcripts, comprising the amino acid sequences of Sequence ID NOS: 24 and 27.

The proteins, fragments and fusion proteins have utility, as described herein, for the preparation of polyclonal and monoclonal antibodies to murine and mammalian Ese proteins, for the identification of binding partners of the mammalian  
20 Ese proteins and for diagnostic and therapeutic methods, as described herein. For these uses, the present invention provides substantially pure proteins, polypeptides or derivatives of polypeptides which comprise portions of the mammalian Ese amino acid sequences disclosed or enabled herein and which may vary from about 4 to 5 amino acids to the complete amino acid sequence of the proteins. The invention  
25 provides substantially pure proteins or polypeptides comprising sequences corresponding to at least 5, preferably at least 10 and more preferably 50 or 100 consecutive amino acids of the mammalian Ese proteins disclosed or enabled herein. Monoclonal antibodies having suitably specific binding affinity for the antigenic regions of a mammalian Ese protein are prepared by the use of corresponding  
30 hybridoma cell lines. In addition, polyclonal antibodies may be prepared by inoculation of animals with suitable peptides which add suitable specific binding affinities for antigenic regions of an Ese protein.

In a further embodiment of the invention, a process is provided for producing mammalian Ese proteins comprising culturing one of the above described transfected  
35 host cells under suitable conditions, to produce the Ese protein by expressing the DNA sequence.

The proteins of the invention may be isolated and purified by any conventional method suitable in relation to the properties revealed by the amino acid sequences of these proteins.

Alternatively, cell lines may be produced which express or over-express the  
5 Ese gene products, allowing purification of the proteins for biochemical characterisation, large-scale production, antibody production and patient therapy.

For protein expression, eukaryotic or prokaryotic expression systems may be generated in which an Ese gene sequence is introduced into a plasmid or other vector which is then introduced into living cells. Constructs in which the Ese cDNA  
10 sequences containing the entire open reading frame is inserted in the correct orientation into an expression plasmid may be used for protein expression. Alternatively, only portions of the sequence may be inserted. Prokaryotic or eukaryotic expression systems allow various important functional domains of the proteins to be recovered as fusion proteins and used for binding, structural and  
15 functional studies and also for the generation of appropriate antibodies.

The present invention includes effective fragments, analogues of the Ese proteins described herein. "Effective" fragments or analogues retain the activity of the described Ese proteins to regulate endocytosis, vesicular trafficking and actin dynamics. The term "analogue" extends to any functional and/or chemical equivalent  
20 of a mammalian Ese protein including mimetics and includes proteins having one or more conservative amino acid substitutions, proteins incorporation unnatural amino acids and proteins having modified side chains.

In accordance with a further embodiment of the invention, antibodies are enabled which bind specifically to the Ese proteins disclosed herein. Polyclonal or  
25 monoclonal antibodies may be prepared using conventional methods. Antibodies may also be prepared to individual selected domains of the Ese proteins, as described herein.

In a further embodiment, the invention provides pharmaceutical compositions containing an Ese protein, fragment or mimetic thereof or a non-functional mutant Ese  
30 protein, fragment or mimetic thereof for the treatment of mammalian disorders which involve abnormal endocytosis, vesicular trafficking and actin dynamics leading to altered cellular functioning. Administration of a therapeutically active amount of a pharmaceutical composition of the present invention means an amount effective, at dosages and for periods of time necessary to achieve the desired result. The  
35 composition comprises an Ese protein and a pharmaceutically acceptable carrier.

In accordance with a further embodiment, the invention provides a method for identifying binding partners of the Ese proteins disclosed herein. Such methods in

general include various assays including those including radiolabelling of the Ese proteins. Other methods may include but are not restricted to phage display, affinity purification techniques, expression cloning and the yeast 2-hybrid system, as described herein.

5 In accordance with a further embodiment of the present invention, is a method for identifying proteins which phosphorylate Ese proteins. Such method includes known phosphorylation assays.

10 The identification of proteins or peptides interact with or bind to Ese proteins can provide the basis for the design of peptide antagonists or agonists of Ese protein function or for the design of peptide antagonists or agonists of Ese protein binding partners which affect Ese protein function. Further, the structure of these peptides determined by standard techniques such as protein NMR or x-ray crystallography can provide the structural basis for the design of improved small molecule drugs.

15 In accordance with a further embodiment, the present invention also provides for the production of mouse models or transgenic non-human animal models for the study of mammalian Ese gene function, for the screening of candidate pharmaceutical compounds, for the creation of *in vitro* mammalian cell cultures which express the Ese proteins or in which an Ese gene has been inactivated by knock-out deletion, and for the evaluation of potential therapeutic interventions.

20 The invention enables a transgenic animal, including a transgenic insect, wherein the genome of the animal or of an ancestor of the animal has been modified by introduction of a transgene comprising mammalian Ese genes under the transcriptional control of tissue restricted regulatory elements including the mouse mammary-tumour virus long term repeat sequences.

25 Transgenic animals with inappropriate expression of Ese proteins may be examined for phenotypic changes, for example abnormal cellular development or abnormal cellular signalling, vesicular trafficking and actin dynamics and may be used to screen for compounds with potential as pharmaceuticals. Compounds which provide reversal of the phenotypic changes are candidates for development as  
30 pharmaceuticals.

Transgenic animals in accordance with the invention can be created by introducing a DNA sequence encoding a selected Ese protein either into embryonic stem (ES) cells of a suitable animal such as a mouse, by transfection or microinjection, or into a germ line or stem cell by a standard technique of oocyte  
35 microinjection. Such methods of producing animal models are fully described in the literature.

In accordance with another aspect of the present invention is a method for screening a candidate compound for effectiveness as an antagonist of an Ese protein comprising:

- 5 (a) providing an assay method for determining the endocytotic regulatory capacity of an Ese protein; and  
(b) determining the endocytotic regulatory capacity of the Ese protein in the presence or absence of the candidate compound, wherein a reduced level of endocytosis in the presence of the candidate compound indicates antagonist activity of the compound.

10 In accordance with another aspect of the present invention is a method for treating in a mammal a disorder associated with an undesired level of endocytotic activity of an Ese protein comprising administering to the mammal an effective amount of a substance selected from the group consisting of:

- 15 (a) an Ese protein antagonist;  
(b) an antibody which binds specifically to an Ese protein;  
(c) an antisense strand comprising a nucleic acid sequence complementary to the sequence or fragment of the sequence and capable of hybridizing to the nucleic acid sequence encoding an Ese protein;  
20 (d) an agent which down regulates the expression of the Ese gene encoding for an Ese protein;  
(e) an antagonist of an Ese protein binding partner; and  
(f) an Ese agonist.

According to another aspect of the present invention is a method for suppressing in a mammal, the abnormal proliferation of a cell capable of being  
25 stimulated to proliferate by a growth factor receptor, the method comprising administering to the mammal an effective amount of a Ese protein antagonist, an Ese agonist or an antibody which binds specifically to an Ese protein.

According to yet another aspect of the present invention is a method for preventing viral infection in a mammal, said method comprising administering to the  
30 mammal an effective amount of an Ese protein antagonist, an Ese agonist or an antibody which binds specifically to an Ese protein or an Ese mutant protein not capable of regulating endocytosis.

According to a further aspect of the present invention is a method for promoting endocytosis, vesicular trafficking and/or actin dynamics in selected cells in  
35 a mammal in need of such treatment, said method comprising administering to the mammal an effective amount of an Ese protein or an active analogue, mimic or fragment thereof.

According to a further aspect of the present invention is a method for blocking clathrin-mediated endocytosis in cultured cells or in selected cells in a mammal in need of such treatment, said method comprising overexpressing Ese1 protein or an  
5 active analogue, mimic or fragment thereof.

According to yet a further aspect of the present invention is a method for regulating endocytosis, vesicular trafficking and/or actin dynamics in cultured cells or in selected cells in a mammal in need of such treatment, said method comprising providing an Ese1-Eps 15 complex and further providing a protein binding partner to  
10 bind to the complex to regulate components of the endocytic pathway. One such binding partner is dynamin.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred  
15 embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

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### **Summary of Tables and Drawings**

A detailed description of the preferred embodiments are provided herein below with reference to the following tables and drawings in which:

25 Table 1 shows alignment of mouse Ese1 and Ese2 protein sequences. EH, coiled-coil and SH3 domains are as indicated.

Table 2 shows the alignment of protein sequences of the Ese family in mouse, Xenopus and Drosophila. EH and SH3 domains are indicated with overlines. Amino acid identities (bolded) and similarity are boxed.

30 Figure 1 shows a Northern blot demonstrating expression of Ese1 and Ese2 genes in various adult tissues.

Figure 2A shows Western blots demonstrating the association of Ese and Eps15 proteins *in vivo*. Endogenous Ese1 proteins were precipitated with Rabbit anti-peptide antisera against the N-terminus of Ese1. Immunoprecipitates were then  
35 analyzed on western blots for the presence of Ese1/2 with Chicken anti-Ese antisera or for co-precipitation of Eps15 with Rabbit antisera raised against the C-terminus of Eps15.



Figure 2B shows alternative transcripts from the Ese1 and Ese2 genes which code for Ese1L and Ese2L proteins respectively, with C-terminal DBL/PH and C2 domains. Ese1 and Ese2 sequence junctions are indicated in bold red letters.

Figure 3A shows Ese and Eps15 associate *in vivo*. This schematic representation of association of Eps15/Eps15R with Ese1 in the yeast two-hybrid screen. Ese1 coiled-coil domain fused with the Gal4 DNA binding domain (DBD) interacted with Eps15/Eps15R Gal4 activation domain (AD) fusions. The AD-Eps15/Eps15R diagram represents the shortest interacting coding region isolated.

Figure 3B shows association of Ese and Eps15 C-terminal deletion mutants. Cos-1 cells were transfected with pcDNA3Eps15, pcDNA3Ese1 or the C-terminal deletion mutants pcDNA3Ese1 $\delta$ C and pcDNA3Eps15 $\delta$ C-Flag as indicated. Cell lysates were immunoprecipitated with rabbit anti-Ese1 or rabbit anti-Eps15 (left panel); rabbit anti-Ese1, mouse anti-Flag or rabbit anti-Eps15 (right panel). Panels represent western blots to detect the presence of Ese1 in each immunoprecipitation. A 90kDa Ese1 protein exists in the third and sixth lanes on the left panel which is the C-terminally truncated Ese1 protein which is co-immunoprecipitated in a complex with Eps15 in the sixth lane. Also, to be noted is the precipitation of Ese1 with anti-Flag monoclonal antibody in the sixth lane of the right panel experiment. In this case, Ese1 has been precipitated in a complex with the C-terminally truncated Eps15 $\delta$ C protein.

Figure 4 shows confocal immunofluorescent micrographs of Cos cells transfected with myc-Ese1 (A, A'); Eps15 (B); myc-Ese1 + Eps15 (C, C', C'') or myc-Ese1 + Eps15 $\delta$ C (D, D' and D''). Frames C'' and D'' represents the overlapping images from frames C/C' and D/D', respectively. Overlap in frames C'' and D'' are indicated in yellow. Scale bar is equal to 10 microns.

Figure 5A and 5B show that Ese1 links to Dynamin and Eps15. Figure 5A is a schematic representation of association between Dynamin and Ese1 in the yeast two-hybrid screen. Ese1 SH3 domains fused with the Gal4 DNA binding domain (DBD) interacted with Dynamin Gal4 activation domain (AD) fusions. The AD-Dynamin diagram represents the shortest interacting coding region isolated. Figure 5B shows confocal immunofluorescent microscopy to detect transfected mycEse1 (frame a), transfected Eps15 (frame d) or endogenous Dynamin (frames b and e) in transfected Cos cells. Frame c and f represents the overlapping images from frames a/b and d/e respectively. Scale bar is equal to 10 microns.

Figure 6 shows Ese1 overexpression blocks endocytosis of Transferrin in Cos-1 cells. Confocal immunofluorescent microscopy was used to detect transfected mycEse1 (frame A), internalized FITC-labeled Transferrin (frame B). Overlapping images are shown in frame C revealing that Ese1 overexpression blocks clathrin-

mediated endocytosis of Transferrin. Mononuclear morphologically normal cells from both transfected and untransfected groups were assessed for internalization of transferrin. Scale bar is equal to 10 microns.

Figure 7 shows a proposed model for Ese regulation of Endocytosis.

5 In the drawings, preferred embodiments of the invention are illustrated by way of example. It is to be expressly understood that the description and drawings are only for the purpose of illustration and as an aid to understanding, and are not intended as a definition of the limits of the invention.

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### **Detailed Description of the Invention**

#### **Sequencing of Murine Ese1 and Ese2 genes**

15 The full length murine Ese1 cDNA was sequenced (Sequence ID NO:1). It encodes a sequence of 1213 amino acids (Sequence ID NO:3) having a predicted molecular weight of 137 kDa. Murine Ese2 cDNA was also sequenced (Sequence ID NO:4) and encodes a sequence of 1197 amino acids (Sequence ID NO:6) having a predicted molecular weight of 135.7 kDa.

20 The full length Ese proteins are predicted to encode two N-terminal EH domains followed by a coiled-coil domain and five SH3 domains (Tables 1 and 2). Several Ese1 isolated clones contain only SH3<sub>A</sub>, SH3<sub>B</sub> and SH3<sub>E</sub> domains. Indeed, the SH3<sub>C</sub> domain was not included in the original human SH3P17 partial cDNA. In addition, expressed sequence tags from the Ese1 gene have been found in the public databases which skip sequences encoding individual EH domains or regions of the coiled-coil domain indicating that this gene is subject to complex alternative splicing and has the potential to encode for many distinct proteins. The predicted Ese2 protein on the other hand encodes a C-terminal extension of 45 amino acids in comparison to the human SH3P18 partial cDNA, suggesting that this gene is also alternatively spliced. The Ese proteins are 53% identical over the full length of Ese1 (645 of 1213 residues in Ese1 line up with identical residues in Ese2) and are related to the Ese protein from *Xenopus* which has recently been submitted to genebank (Accession # AF032118) and *Drosophila* (59) (Table 2). *Xenopus* Intersectin is 81% identical to mouse Ese1 and 54% identical to mouse Ese2 suggesting that Intersectin is an Ese1 orthologue (980/1213 residues of mouse Ese1 and 645/1198 residues of mouse Ese2 line up with identical residues in the *Xenopus* protein). *Drosophila* Dynamin associated protein, Dap160-1 is 32% identical to both mouse Ese proteins (393/1213 residues of mouse Ese1 and 387/1198 residues of mouse Ese2 line up with identical residues in the *Drosophila* protein). These homologies extend over the entire length

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of Dap160-1, except that Dap160-1 has only four SH3 domains corresponding to the first, second, fourth and fifth SH3 domains of the mouse Ese proteins. Additional sequence analysis reveals the presence of a very large number of potential phosphorylation sites and at least one SH3-binding consensus (60) in the N-terminus of each Ese protein.

The two EH domains of Ese1 are highly related to the respective EH domains in Ese2 and these Ese EH domains are most closely related those found in Eps15 and Eps15R, two mammalian protein which are required for endocytosis through clathrin-coated pits. EH domains have also been identified in End3 and Pan1p which are yeast partners proteins required for endocytosis. The central third of both Ese proteins are predicted to encode an extended coiled-coil which is a domain typically associated with protein-protein association through dimerization or tetramerization as noted for Eps15 (29, 54). Besides the homology between Ese1 and Ese2, the Ese1 SH3 domains are most closely related to SH3 domains from Myosin IB in Acanthamoeba (SH3<sub>A</sub>), Myosin IB in Entamoeba (SH3<sub>B</sub>), the YFR024 hypothetic yeast protein (SH3<sub>C</sub>), Myosin IB from Acanthamoeba (SH3<sub>D</sub>) and Myosin IC from Acanthamoeba (SH3<sub>E</sub>). The same homologies are noted for SH3 domains from Ese2 with the exception of SH3<sub>A</sub> which is most similar to the SH3 domain from  $\beta$ PIX, SH3<sub>C</sub> which is most similar to an SH3 domain from the mouse Ray protein and SH3<sub>D</sub> which is most similar to the SH3 domain from Dictyostelium myosin IB. Additional sequence analysis reveals the presence of a very large number of potential phosphorylation sites in the Ese proteins and a single SH3 binding consensus in the N-termini of each Ese protein.

With the knowledge of the amino acid sequences for Ese1 and Ese2 proteins and the alternative transcripts Ese1L and Ese2L, there is provided in accordance with the present invention antibodies which recognize epitopes within these proteins and which can be raised to provide information on the characteristics of the protein as well as for any mutant form of these proteins. The generation of antibodies enables the visualization of the protein in mammalian cells and tissues using Western blotting as described herein. Antibodies to the Ese1 or Ese2 proteins also allows for the use of immunocytochemistry and immunofluorescence techniques in which the proteins are visualized directly in cells and tissues as described herein. This is most helpful in order to establish the subcellular location of the protein and the tissue specificity of the protein.

In general, methods for the preparation of antibodies are well known. In order to prepare polyclonal antibodies, fusion proteins containing defined portions or all of

the Ese1 or Ese2 proteins or any of their alternative transcripts can be synthesized in bacteria by expression of corresponding DNA sequences in a suitable cloning vehicle. The protein can then be purified, coupled to a carrier protein and mixed with Freund's adjuvant (to help stimulate the antigenic response by the rabbits) and injected into rabbits or other laboratory animals. Alternatively, protein can be isolated from cultured cells expressing the protein. Following booster injections at bi-weekly intervals, the rabbits or other laboratory animals are then bled and the sera isolated. The sera can be used directly or purified prior to use, by affinity chromatography. The sera can then be used to probe protein extracts run on a polyacrylamide gel to identify the Ese1 or Ese2 protein, alternative transcript or any mutant thereof. Alternatively, synthetic peptides can be made to the antigenic portions of these proteins and used to inoculate the animals.

Methods to produce monoclonal antibodies which specifically recognize mammalian Ese1 or Ese2 proteins or portions thereof, are known in the art. In general, cells actively expressing the protein are cultured or isolated from tissues and the cell extracts isolated. The extracts or recombinant protein extracts, containing the Ese1 or Ese2 protein, are injected in Freund's adjuvant into mice. After being injected 9 times over a three week period, the mice spleens are removed and resuspended in phosphate buffered saline (PBS). The spleen cells serve as a source of lymphocytes, some of which are producing antibody of the appropriate specificity. These are then fused with a permanently growing myeloma partner cell, and the products of the fusion are plated into a number of tissue culture wells in the presence of a selective agent such as HAT. The wells are then screened to identify those containing cells making useful antibody by ELISA. These are then freshly plated. After a period of growth, these wells are again screened to identify antibody-producing cells. Several cloning procedures are carried out until over 90% of the wells contain single clones which are positive for antibody production. From this procedure a stable lines of clones is established which produce the antibody. The monoclonal antibody can then be purified by affinity chromatography using Protein A or Protein G Sepharose.

The Ese proteins may be isolated and purified by methods selected on the basis of properties revealed by its sequence. Purification can be achieved by protein purification procedures such as chromatography methods (gel-filtration, ion-exchange and immunoaffinity), by high-performance liquid chromatography (HPLC, RP-HPLC, ion-exchange HPLC, size-exclusion HPLC, high-performance chromatofocusing and hydrophobic interaction chromatography) or by precipitation (immunoprecipitation).

Polyacrylamide gel electrophoresis can also be used to isolate the Ese proteins based on the molecular weight of the protein, charge properties and hydrophobicity.

Similar procedures to those mentioned can be used to purify the protein from cells transfected with vectors containing an Ese gene (e.g. baculovirus systems, yeast expression systems and eukaryotic expression systems).

The purified proteins can be used in further biochemical analyses to establish secondary and tertiary structure which may aid in the design of pharmaceuticals to interact with the protein, alter the protein charge configuration or charge interaction with other proteins or alter its function.

The Ese proteins can also be purified from the creation of fusion proteins which are expressed and recovered from prokaryotic or eukaryotic cells. The fusion proteins can be purified by affinity chromatography based upon the fusion vector sequence. The Ese protein can then be further purified from the fusion protein by enzymatic cleavage of the fusion protein.

#### Expression of Ese genes

In order to determine where the Ese genes are expressed Northern analysis was performed on mRNA derived from several adult mouse tissues (Figure 1). These genes are both widely expressed and reveal a complex pattern of alternatively spliced transcripts. The highest levels of Ese1 mRNA were noted in brain, heart and skeletal muscle. Brain and skeletal muscle mRNA also contain an unusually large transcript which may be as much as 15kb in length. Interestingly, these tissues express high levels of many proteins involved in synaptic vesicle recycling and endocytosis.

The Northern blot reveals the presence of many alternative mRNA transcripts for both Ese1 and Ese2. In addition, numerous Ese1 expressed sequence tags have been identified which skip sequences encoding individual EH domains or regions of the coiled-coil domain indicating that this gene has the potential to encode for many distinct proteins. Indeed, the SH3<sub>C</sub> domain was spliced out of the human SH3P17 partial cDNA described by Sparks 1996 (53). The predicted Ese2 protein on the other hand encodes a C-terminal extension of 45 amino acids in comparison to the human SH3P18 partial cDNA, revealing that this gene is also alternatively spliced. Lung, liver and kidney express high levels of a 2.4kb Ese2 transcript which is significantly smaller than the 3591bp sequence required to code for our full Ese2 protein as shown in Table 1 or 2, indicating that a major isoform of Ese2 has only a subset of the domains described above.

### Alternative Splicing of Ese1, Ese2 transcripts

Additional transcripts from both Ese1 and Ese2 genes have also been characterized. One of the Ese1 clones isolated from a mouse brain cDNA library contained an extended reading frame. PCR was used from mouse brain cDNA  
5 libraries to identify the remaining coding sequences from this transcript. The splicing event which produces this extended protein adds an additional 501 amino acids to Ese1, and codes for a DBL homology domain, a Pleckstrin homology (PH) domain and a C2 domain (Figure 2B). DBL and PH domains are found together in guanine  
10 nucleotide exchange factors for the Rho family of small GTPases. This long form of Ese1 is therefore expected to function as an activator of Rho-GTPases, which in turn regulate the actin cytoskeleton and numerous signal transduction pathways (61). C2 domains on the other hand are  $\text{Ca}^{++}$  activated membrane binding domains and protein-protein interaction domains (62). A number of expressed sequence tags which code for a novel C2 domain fused to the sequence coding for the last 11 amino acids of  
15 Ese2 have been identified. PCR was used to isolate sequences coding for alternatively spliced exon(s) which can be included C-terminal to the SH3 domains but before the stop codon. The alternatively spliced exon(s) of Ese2 also code for a DBL/PH + C2 domain cassette which can be included within the Ese2 transcript (Figure 2B).

As the Ese genes are subject to complex alternative splicing to produce  
20 proteins with novel predicted functions (eg. regulation of the cytoskeleton and membrane-binding), the alternative proteins are named as modifications of Ese. The Ese1 and Ese2 long forms described in Figure 2B are called Ese1L and Ese2L, respectively. In the event that specific domains are spliced out from the transcripts coding for Ese1 and Ese2 proteins as shown in Table 2, then the name is listed as an  
25 Ese $\delta$  variant. The protein encoded by the spliced variant in the original SH3P17 clone is designated as Ese1 $\delta\text{S}_\text{C}$  to indicate removal of SH3 $\text{C}$ .

In an embodiment of the present invention the knowledge of the Ese1 and Ese2 gene sequences and their expression in heterologous cell systems can be used to demonstrate structure-function relationships as well as provide for cell lines for the  
30 purposes of drug screening. Ligating the Ese1 or Ese2 cDNA sequence into a plasmid expression vector to transfect cells is a useful method to test the proteins influence on various cellular biochemical parameters including the identification of substrates, binding partners as well as activators and inhibitors of the proteins. Plasmid expression vectors containing either the entire, or portions thereof, Ese1 or Ese2 can  
35 be used in *in vitro* mutagenesis experiments which will identify portions of the protein crucial for regulatory function.

The Ese1 or Ese2 cDNA sequence (or Ese1L and Ese2L cDNA sequence) can

be manipulated in studies to understand the expression of the gene and its product, to achieve production of large quantities of the protein for functional analysis, for antibody production, and for patient therapy. The changes in the sequence may or may not alter the expression pattern in terms of relative quantities, tissue-specificity and functional properties. Partial or full-length cDNA sequences which encode for the Ese1 or Ese2 protein (or alternative transcripts thereof), modified or unmodified, may be ligated to bacterial expression vectors. *E. coli* can be used using the T7 RNA polymerase/promoter system using two plasmids or by labeling of plasmid-encoded proteins, or by expression by infection with M13 Phage mGPI-2. *E. coli* vectors can also be used with Phage lambda regulatory sequences, by fusion protein vectors (eg. lacZ and trpE), by maltose-binding protein fusions, and by glutathione-S-transferase fusion proteins.

Alternatively, the Ese 1 or Ese2 protein or alternative transcripts thereof can be expressed in insect cells using baculoviral vectors, or in mammalian cells using vaccinia virus. For expression in mammalian cells, the cDNA sequence may be ligated to heterologous promoters, such as the simian virus (SV40) promoter in the pSV2 vector and introduced into cells, such as COS cells to achieve transient or long-term expression. The stable integration of the chimeric gene construct may be maintained in mammalian cells by biochemical selection, such as neomycin, G418 and purimycin.

Any of the Ese1 or Ese2 cDNA sequences can be altered using procedures such as restriction enzyme digestion, fill-in with DNA polymerase, deletion by exonuclease, extension by terminal deoxynucleotide transferase, ligation of synthetic or cloned DNA sequences, site-directed sequence alteration with the use of specific oligonucleotides together with PCR.

The cDNA sequence or portions thereof, or a mini gene consisting of a cDNA with an intron and its own promoter, is introduced into eukaryotic expression vectors by conventional techniques. These vectors permit the transcription of the cDNA in eukaryotic cells by providing regulatory sequences that initiate and enhance the transcription of the cDNA and ensure its proper splicing and polyadenylation. The endogenous Ese1 or Ese2 gene promoter can also be used. Different promoters within vectors have different activities which alters the level of expression of the cDNA. In addition, certain promoters can also modulate function such as the glucocorticoid-responsive promoter from the mouse mammary tumor virus.

Some of the vectors listed contain selectable markers or neo bacterial genes that permit isolation of cells by chemical selection. Stable long-term vectors can be maintained in cells as episomal, freely replicating entities by using regulatory

elements of viruses. Cell lines can also be produced which have integrated the vector into the genomic DNA. In this manner, the gene product is produced on a continuous basis.

5 Vectors are introduced into recipient cells by various methods including calcium phosphate, electroporation, lipofection, DEAE dextran, microinjection, or by protoplast fusion. Alternatively, the cDNA can be introduced by infection using viral vectors.

10 Eukaryotic expression systems can be used for many studies of the Ese1 or Ese2 gene and gene product(s) including determination of proper expression and post-translational modifications for full biological activity, identifying regulatory elements located in the 5' region of the Ese1 or Ese2 gene and their role in tissue regulation of protein expression, production of large amounts of the normal and mutant protein for isolation and purification, to use cells expressing the Ese1 or Ese2 protein or  
15 alternative transcripts thereof as a functional assay system for antibodies generated against the protein or to test effectiveness of pharmacological agents, or as a component of a signal transduction system, to study the function of the normal complete protein, specific portions of the protein, or of naturally occurring and artificially produced mutant proteins.

20 Using the techniques mentioned, the expression vectors containing the Ese1 or Ese2 cDNA sequence or portions thereof can be introduced into a variety of mammalian cells from other species or into non-mammalian cells. It is understood that the cDNA sequences for use in the present invention include those sequences disclosed herein encoding Ese1, Ese1L, Ese2 and Ese2L proteins.

25 The recombinant cloning vector, according to this invention, comprises the selected DNA of the DNA sequences of this invention for expression in a suitable host. The DNA is operatively linked in the vector to an expression control sequence in the recombinant DNA molecule so that Ese1 or Ese2 proteins or alternative transcripts thereof can be expressed. The expression control sequence may be selected from the group consisting of sequences that control the expression of genes  
30 of prokaryotic or eukaryotic cells and their viruses and combinations thereof. The expression control sequence may be selected from the group consisting of the lac system, the trp system, the tac system, the trc system, major operator and promoter regions of phage lambda, the control region of the fd coat protein, early and late promoters of SV40, promoters derived from polyoma, adenovirus, retrovirus, baculovirus, simian virus, 3-phosphoglycerate kinase promoter, yeast acid  
35 phosphatase promoters, yeast alpha-mating factors and combinations thereof.

The host cell which may be transfected with the vector of this invention may



be selected from the group consisting of *E.coli*, *pseudomonas*, *bacillus subtilis*, *bacillus stearothermophilus*, or other bacilli; other bacteria, yeast, fungi, insect, mouse or other animal, plant hosts, or human tissue cells.

#### 5 Subcellular localisation of Ese proteins

To determine the subcellular localization of Ese1, a myc-epitope tagged version of this protein (mycEse1) was engineered. This tagged protein was expressed in Cos cells and detected by confocal immunofluorescence using the mouse anti-myc monoclonal antibody 9E10 (Figure 4). Interestingly, the transfected Ese1 protein is  
 10 highly concentrated into circular domains (Figure 4A and 4A') which are present throughout transfected cells (compare single optical section in Figure 4A with the projection of all sections through the same cell shown in 4A'). In some optical sections rings of fluorescent staining surrounding a non-staining area were observed suggesting that the Ese1 induced structures are vesicles rather than inclusion bodies  
 15 (data not shown and Figure 6). Ese1 overexpressed in 10T1/2, BHK and Hela cells using the vaccinia virus T7 expression system is also localized to large circular domains (data not shown). This concentration of ectopically expressed Ese1 contrasts with the localization of Eps15 in transiently transfected cells, Eps15 being dispersed throughout the cell (Figure 4B). Ese1 and Eps15 proteins form a complex *in vivo* and yet localize to distinct subcellular compartments in transfected Cos cells. We  
 20 therefore determined the localization of both mycEse1 and Eps15 in Cos cells co-transfected with both genes. In co-transfected cells, mycEse1 is still found in circular domains (Figure 4C). Interestingly, the transfected Eps15 is now also partially co-localized with Ese1 in the same circular domains (Figure 4C' and C'').  
 25 The C-terminal third of Eps15 contains several regions which are required for association with  $\alpha$ -adaptin of the AP2 clathrin adaptor complex (31). It has been shown that this region of Eps15 is not required for its association with Ese1 (Figure 4). In order to test whether Eps15 function may be required for Ese localization, mycEse1 and Eps15 $\delta$ C have been co-transfected. Interestingly, mycEse1 localization  
 30 is still partially colocalized with Eps15 $\delta$ C (Figure 4D, D' and D'') but is no longer found to be concentrated within circular structures, indicating that Ese1 requires Eps15 function for distinctive subcellular localization in transfected cells. In addition, these data suggest that the Ese1:Eps15 complex may require association with AP2 which binds to the Eps15 C-terminus in order to form the large circular  
 35 domains in Ese1 transfected cells.

### Identification of binding partners of Ese proteins

In order to identify Ese partners, a systematic analysis was initiated of each domain for binding partners using the yeast two hybrid system. The central coiled-coil domain of Ese1 from amino acid 330 to 732 was fused to the GAL4 DNA binding domain and transformed into the Y190 reporter strain together with plasmid cDNA libraries from several tissue sources. Yeast colonies were selected for growth on Histidine minus plates in the presence of 40mM 3-amino triazole to select for interaction between library encoded GAL4 activation domain fusions and the GAL4 DNA binding domain Ese1 coiled-coil bait. Yeast colonies which survived selection for expression of the His3 gene were also tested for induction of the integrated LacZ gene which is GAL4 responsive in Y190. Prey plasmids were recovered from 37 positive yeast colonies which were selected for expression of both His3 and LacZ. Several classes of cDNA were recovered in this screen. One class included Eps15 which was isolated twice and Eps15R which was isolated from four yeast colonies. Interestingly these clones were all partial cDNA fusions which minimally included C-terminal sequences from the central coiled-coil domain to the 3'UTR (Figure 3A). In the case of Eps15, the positive clones start from amino acid 306 and 376 whereas in Eps15R the N-terminal boundary of clones were amino acid 4, 10, 222 and 386. These data indicate that minimal sequences required for interaction between the Ese1 coiled-coil domain and Eps15(R) include either or both of the central coiled-coil and/or the C-terminal third of these proteins.

The Ese proteins were analyzed *in vivo* to test for their association with Eps15 or Eps15R. Polyclonal antisera were raised in chickens against a GST fusion containing the C-terminus of Ese1 from amino acid 665 to the stop codon. This region of Ese1 contains all five of the SH3 domains. In addition, polyclonal antisera were generated in rabbits against a peptide representing the first 21 amino acids of Ese1. Cell lysates were prepared from A431, PC12, MDCK and Hela cells which represent cell lines from several distinct tissue types and species. The rabbit anti-peptide antisera were used to precipitate Ese1 from each lysate and precipitates were analyzed by western blotting using the chicken anti-Ese sera. In each cell line the presence of several specific bands in the range of 150kDa were observed which were precipitated in the absence but not in the presence of the peptide to which the sera was generated (Figure 3A). Thus the Ese1 protein is expressed in many tissue culture cells and runs in a range consistent with the predicted molecular weight of 137kDa. The same samples were also analyzed for co-immunoprecipitation of Eps15 proteins. In each case multiple Eps15 proteins were detected which co-purify with Ese1. The anti-Ese1 peptide antisera and commercially available anti-Eps15 antisera which were

used were raised against epitopes which are not shared by these proteins indicating that Ese1 and Eps15 are constitutively associated partners *in vivo*. This association is reminiscent of the previously detected complex between two EH domain containing yeast proteins, End3p and Pan1p.

5        Ese1 and Eps15 proteins both contain central coiled-coil motifs. In addition, Ese1 contains multiple C-terminal SH3 domains while Eps15 contains SH3-binding motifs. In order to map the regions of each protein which are required for their association *in vivo*, C-terminal truncations of each (Eps15 $\delta$ C and Ese1 $\delta$ C) were generated. Full length Eps15 was co-transfected into Cos-1 cells together with either  
10       full length Ese1 or C-terminally truncated Ese1 $\delta$ C. Cell lysates were precipitated with either rabbit anti-Eps15 or with rabbit anti-Ese1, and precipitates were western blotted with chicken anti Ese1 antisera. Interestingly, the C-terminally truncated Ese1 protein was efficiently immunoprecipitated in a complex with Eps15 using anti-Eps15 sera. In a reciprocal experiment Cos-1 cells were transfected with Ese1 alone or together  
15       with Eps15 or C-terminally truncated Eps15 which had been Flag-epitope tagged (Eps15 $\delta$ C-Flag). Cell lysates were prepared and immunoprecipitated with either rabbit anti-Ese1, mouse anti-Flag, or rabbit anti-Eps15 antibodies. These immunoprecipitations were also western blotted to analyze for the presence of Ese1 (Figure 3B). The anti-Flag antibody efficiently precipitated Ese1 from cells  
20       expressing Flag-tagged Eps15 $\delta$ C indicating that the C-terminally truncated Eps15 protein can bind to Ese1 *in vivo*. The Ese1 and Eps15 proteins are therefore associated through interaction of their central coiled-coil regions and do not require the presence of SH3 and SH3-binding motifs in their respective C-termini.

In addition to the identification of Eps15 and Eps15R in the yeast two hybrid  
25       screen with the coiled-coil domain bait of Ese1, the following were also identified as Ese binding proteins: TSG101 (accession #U52945), meningioma expressed antigens 6/11 (accession #U94780 for mea6),  $\beta$ -tropomyosin, rabaptin5 (accession #D86066), Adora2a (accession #Y13345), L1 lipid binding protein (accession #K02109) and numerous cytokeratins and laminins. Novel genes identified in this screen are  
30       detailed below.

Also performed was a yeast two hybrid screen using amino acids 665-1213 of Ese1 as bait. This screen led to the isolation of the following clones which produced GAL4 Activation domain fusions which bound to this SH3 domain bait of Ese1. The Ese-binding proteins identified in our SH3 screen were the cbl-b oncoprotein  
35       (accession #U26712), Dynamin II (accession #L31398), KIAA0268 (accession #D87742), Jerky (accession #U35730), hnRNP-K (accession #L29769), SAP49

(accession #L35013) and SOS-1 (accession #Z11574). Novel genes identified in this screen are detailed below and several novel clones as outlined below.

These results demonstrate that the novel Ese genes and the proteins that they encode function in a complex with Eps15 proteins to regulate endocytosis together. In addition, this complex contains binding sites for numerous other proteins. Furthermore, with the identification of several potential phosphorylation sites on the Ese proteins, these results also suggest that whether or not complexed with Eps15, Ese proteins are involved in intracellular signalling processes which are likely to lead to altered cellular activity. Many Ese partners have been identified in these studies. Also revealed is a novel method to identify more Ese partners. Yeast cells have a complex formed by two EH domain proteins (Pan1p and End3p) which regulates both endocytosis as well as the actin cytoskeleton. Indeed, the Eps15 protein has been reported to regulate both endocytosis and the actin cytoskeleton (1). As Eps15 and Ese function together, and Ese contain many protein-protein interaction surfaces on this complex, strongly suggesting that the Ese proteins and their binding proteins are critical regulators of Eps15:Ese functions *in vivo*.

#### Ese and Dynamin

Yeast two-hybrid screens using baits composed of the GAL4 DNA-binding domain fused to individual SH3 domains of Ese1 were performed to identify Ese partners which bind the SH3s of this multi-domain protein. Initial screens with SH3B and SH3C domain fusions resulted in the artifactual isolation of many proline-rich fragments which did not represent real Ese partners. Screens were then done with GAL4 fusions containing all five SH3 domains from amino acid 665 to 1213. Forty one His3+/LacZ+ colonies from such screens were selected for further analysis. Six colonies were found to encode fragments of the Dynamin II gene and two encoded fragments of Dynamin I (Figure 5A). All Dynamin II clones had 5' start sites between amino acid 252 and 278 and terminated within the 3' UTR. The two Dynamin I clones were identical and contained a small coding region from amino acid 673 to the C-terminus. Dynamin sequences contained within each Ese1-interacting clone therefore minimally code for the proline-rich motifs (Figure 5A) which are known to bind SH3 domains *in vitro* (44). This result is consistent with the interaction between Dap160 and Dynamin that has been recently described in *Drosophila* (59). To determine the significance of Ese1:Dynamin binding, functional interactions between these proteins were tested for. MycEse1 was transfected into Cos cells. The subcellular localization of Dynamin in Ese1 overexpressing cells was analyzed by staining for endogenous Dynamin and the myc tag on Ese1. In many

Ese1 transfectants, endogenous Dynamin was recruited to the Ese1 staining vesicles and both proteins were co-localized (Figure 5B, panel a, b and c). These results demonstrate that Ese proteins bind Dynamin and can regulate its subcellular localization. Given that Ese1 functions in a complex with Eps15 and Ese can regulate the subcellular distribution of Dynamin, it was investigated whether Eps15 also co-localized with Dynamin. Eps15 transfected into Cos cells was diffusely localized (Figure 4). Endogenous Dynamin was not concentrated and was therefore not visible in Eps15 transfected cells (data not shown). When Ese1 and Eps15 were co-transfected however, Eps15 and endogenous Dynamin are colocalized on the Ese1 induced circles (Figure 5, panels d, e and f) indicating that all three proteins co-localize at these structures.

#### Ese and Epsin Family Proteins

In order to identify additional Ese partners, a fusion between GAL4-DBD and the N-terminal EH domain region of Ese1 (amino acids 1-393) was generated. This fusion protein was also used in yeast two hybrid screens with several GAL4 activation domain cDNA libraries. A total of 11 His3+/LacZ+ colonies were identified as containing library encoded fusion proteins which interact with the Ese1 EH-domain bait. Two of these clones coded for mouse homologues of the Epsin protein which was recently identified on the basis of its affinity for Eps15, and  $\alpha$ -adaptin and is required for clathrin-mediated endocytosis (63). The two Epsin clones code for C-terminal fragments from amino acids 403 and 470 respectively. One of the Ese1 EH-domain interacting clones which was obtained coded for a C-terminal fragment of a novel Epsin-family protein (63, 64). This cDNA has been independently isolated and named Ibp-2 in accession #AF057286. The Ibp2 clone which has been identified includes amino acids 326 to 509 of the partial coding sequence in Genbank. Each Epsin family protein which has been isolated contains a C-terminal region which codes for three copies of an NPF motif. This is also the region of Epsin which binds to Eps15. It has previously been shown that AP-2 can independently bind both Epsin and Eps 15. It is now demonstrated that the C-terminus of Epsin family proteins can bind not only to the EH domains of Eps15 but also to the EH domains of Ese1. These data suggest that either multiple Epsin proteins exist in the Ese:Eps15 complex or the interaction between individual proteins in various AP-2:Epsin:Eps15:Ese:Dynamin complexes may be subject to dynamic rearrangement during clathrin coated pit formation, invagination and scission. Also identified was a mammalian homologue of *drosophila*, SINA, seven in absentia.

### Ese1 Overexpression Blocks Endocytosis

Overexpression of Pan1p in yeast induces phenotypes which are identical to those observed in pan1 loss-of-function mutants (65) suggesting that by altering the relative ratios of Pan1p to its numerous partners, the function of higher order Pan1p complexes may be blocked. As the Ese:Eps15 complex contains many protein-protein interaction domains which bind partners including Dynamin, Epsin and AP-2, it was thought that overexpressed Ese1 may disrupt the formation of higher order complexes between Ese proteins and partners. It was therefore tested whether clathrin-mediated endocytosis was functional in myc Ese1 overexpressing cells. Cos-1 cells were transfected with myc-tagged Ese1, and 48 hrs post transfection, FITC-labelled Transferrin was added to cultures for 30 minutes. Cells were then fixed and analyzed for expression of mycEse1 and for internalization of Transferrin. As shown in Figure 6, transfected cells do not internalize transferrin in contrast to their untransfected neighbors. Cell counts in a representative experiment indicate that 96% of Ese1 overexpressing cells do not internalize labeled transferrin (N=46) whereas 100% of untransfected cells were capable of clathrin-mediated endocytosis (N=100). It appears that overexpression of Dynamin II may override the Ese1 induced endocytic block. These data indicate that overexpression of Ese1 blocks endocytosis and this may be through sequestration of Dynamin or other Ese1 partners into non-productive binary complexes during endocytosis or recycling of the transferrin receptor. Overexpressed Ese1 would therefore be functioning as a dominant inhibitory protein through recruitment of partners into non-productive complexes which do not contain all of the necessary components for endocytosis to proceed.

Taken together, these studies demonstrate the identification and isolation of novel mammalian Ese proteins encoded by novel Ese genes which are involved in the regulation of endocytosis via clathrin-coated pits, vesicular trafficking and actin dynamics. These studies also demonstrate the identification and isolation of two alternative transcripts of the genes, named Ese1L and Ese2L. The process of endocytosis including receptor-mediated endocytosis as well as pinocytosis or non-receptor mediated endocytosis. The novel Ese proteins of the present invention appear to function to regulate endocytosis involving the formation of clathrin-coated pits by the polymerization of clathrin into a lattice along the cytosolic face of a region of membrane causing the region to expand inward. Ultimately, the pit pinches off from the membrane, and the clathrin cage is completed thus producing a coated vesicle. Through the binding of Eps15 to form an Ese-Eps15 complex via a central binding

region, the complex can recruit other proteins such as, Epsin, AP-2 and dynamin and promote coated vesicle formation and perhaps their transport to appropriate locations within the cell and release of internalized proteins and/or molecules (Figure 7). The SH3 C-terminal domains of the Eps proteins can bind and interact with several other proteins leading to a host of protein-protein interactions involved in subcellular trafficking and signalling. The Eps proteins may be activated or inactivated via phosphorylation of the proteins at numerous phosphorylation sites by the action of activated receptors on cell surfaces.

Due to the fact that the Eps proteins appear to be a key central player in the complex process of endocytosis involving protein-protein interactions and intracellular signalling, these proteins are most likely involved in a myriad of clinical conditions and processes which are very likely to include but not be limited to regulation of endocytosis (as described above), cell division and cancer (Eps15 and cbl are oncoproteins), cell migration (regulation of the actin cytoskeleton is required for many forms of cell migration), cell polarity, plane of cell division and cell fate specification (Eps15 binds to Numb *in vivo* which is required for these processes (43), RNA localization (several RNA binding proteins have been identified in the present screens) and viral infection and life cycle (Eps15 binds to RAB a cellular cofactor for HIV Rev (43)).

With respect to viral infection Eps proteins may play an important role and thus may be a target for developing therapeutic strategies against viral infection and virally-induced disease states. HIV is known to alter endocytosis of several important cell surface molecules including CD4 and MHC antigens. HIV-NEF has been demonstrated to bind to SH3 domains. NEF induces clathrin coated pit formation. As NEF binds SH3 domains and induces endocytosis, it is possible that Eps proteins may bind to NEF and are involved in NEF function. Therapeutic strategies to provide treatment for viral infection and virally induced disease states may therefore include the inhibition of Eps-NEF interactions, antibodies or other agents directed against Eps complexes to inhibit endocytosis and in this manner inhibit viral infection and virally induced disease states. It is also expected that many types of viruses will interact with the multi-component Eps15-Eps complex.

Synaptic transmission and abnormal or altered synaptic transmission as seen in various nervous disorders may also be a target for the therapeutic use of Eps proteins and/or antagonists. The Eps15:Eps complex has been demonstrated to regulate endocytosis, is highly expressed in the brain and Eps binds to Jerky; a protein required to prevent epilepsy in mice (57). Furthermore, Eps proteins are highly expressed not only in the brain but also in the heart and in skeletal muscle which are tissues

involving high levels of synaptic transmission. This suggests that Ese protein may be used in the treatment of nervous system disorders involving altered synaptic transmission.

Receptor-mediated cell signaling such as seen with several different types of growth factors also involves endocytosis. Over-expression, mutation or over-stimulation of growth factor receptors has been demonstrated to lead to abnormal cell division and growth as seen in cancer. For example, EGF is a potent mitogen for many epithelial cells and EGF receptor activation is known to stimulate intracellular kinase pathways leading to cell proliferation. Such activity may play a role in cancer progression. By altering the rate of endocytosis by targeting Ese proteins, the cell proliferative effect of growth factor receptor stimulation may be counteracted.

Abnormal cell division and cell migration is seen in several diseases and involves the cell cytoskeleton. The intracellular cytoskeleton is highly organized and consists of microtubules, microfilaments and intermediate filaments acting as an internal reinforcement in the cytoplasm of a cell. Together these structures associate in a regular and defined manner which is regulated by extracellular signals and may transduce plasma membrane signals by association with other proteins or by second messengers. The Ese-Eps15 complex is very likely to regulate the cytoskeleton by analogy to the role of Pan1p:End3p in regulation of yeast cytoskeleton. Furthermore, endocytosis is known to involve a rearrangement of the intracellular cytoskeleton. Cell division and migration require the continual rearrangement of the intracellular cytoskeleton. Therefore, abnormal patterns of cell division and migration may involve altered Ese function and altered endocytosis. The Ese proteins or the genes may therefore be used to alter regulation of endocytosis or the association of the Ese proteins with the cytoskeleton and may restore cell division and migration to normal levels and patterns.

Tissue development also involves the continual remodeling of the cytoskeletal network along with its associated proteins. Developmental diseases can occur as a result of abnormal remodeling of the cytoskeleton leading to altered intracellular signaling. As Ese proteins are likely to be involved with both the cytoskeleton and intracellular signaling they may also be directly involved in the development of certain developmental diseases and therefore may be a target for therapeutic treatment of such diseases. Ese proteins may also be involved in normal development including that of stem cells which are self-renewing cells that divide to produce differentiated daughter cells in various tissues. As Ese proteins are associated with the cytoskeleton they may play a part in the formation of certain types of differentiated cells through the partitioning of RNA and proteins such as nuMb during cell division.



Transgenic Animal Models

5 The creation of transgenic animal models for abnormal endocytotic function characterized by altered Ese1 or Ese2 activity is important to the understanding of the function of these proteins in intracellular signaling and for the testing of possible therapies for abnormal endocytosis involving protein-protein interactions and intracellular signalling and leading to various clinical conditions. In general, techniques of generating transgenic animals are widely accepted and practiced

10 There are several ways in which to create an animal model in which the Ese1 or Ese2 gene expression or function is altered. One could simply generate a specific mutation in the mouse Ese1 or Ese2 gene as one strategy. Secondly a wild type human Ese1 or Ese2 gene and/or a humanized murine gene could be inserted into the animals genome by homologous recombination. It is also possible to insert a mutant (single or multiple) human gene as genomic or minigene construct using wild type or mutant or artificial promoter elements. More commonly, and most preferred in the present invention, knock-out of the endogenous murine genes may be accomplished by the insertion of artificially modified fragments of the endogenous gene by homologous recombination. The modifications include insertion of mutant stop codons, the deletion of DNA sequences, or the inclusion of recombination elements (lox p sites) recognized by enzymes such as Cre recombinase. Gene knockout produces homozygous mutant mice, which show symptoms or phenotype similar to those exhibited by a human.

25 In general, for gene knock-out, embryonic stem cells heterozygous for a knockout mutation in a gene of interest (ie. Ese1 or Ese2 gene) and homozygous for a marker gene (eg. coat colour) are transplanted into the blastocoel cavity of 4.5 day embryos homozygous for an alternate marker. The early embryos then are implanted into a pseudopregnant female. Some of the resulting progeny are chimeras. Chimeric mice then are backcrossed. Intercrossing will eventually produce individuals homozygous for the disrupted allele that is, knockout mice. (Capecchi, MR. 1989. Science. 244:1299-1291).

30 To inactivate the Ese1 or Ese2 mouse gene chemical or x-ray mutagenesis of mouse gametes, followed by fertilization, can be applied. Heterozygous offspring can then be identified by Southern blotting to demonstrate loss of one allele by dosage, or failure to inherit one parental allele using RFLP markers.

35 To create a transgenic mouse, a mutant or normal version of the human Ese1 or Ese2 gene can be inserted into a mouse germ line using standard techniques of oocyte microinjection or transfection or microinjection into stem cells. Alternatively,

if it is desired to inactivate or replace the endogenous Ese1 or Ese2 gene, homologous recombination using embryonic stem cells may be applied.

For oocyte injection, one or more copies of a mutant or normal Ese1 or Ese2 gene can be inserted into the pronucleus of a just-fertilized mouse oocyte. This oocyte is then reimplanted into a pseudo-pregnant foster mother. The liveborn mice can then be screened for integrants using analysis of tail DNA for the presence of transgenic Ese1 or Ese2 gene sequences. The transgene can be either a complete genomic sequence injected as a YAC or chromosome fragment, a cDNA with either the natural promoter or a heterologous promoter, or a minigene containing all of the coding region and other elements found to be necessary for optimum expression.

Retroviral infection of early embryos can also be done to insert the human or mouse Ese1 or Ese2 gene. In this method, the Ese1 or Ese2 gene is inserted into a retroviral vector which is used to directly infect mouse embryos during the early stages of development to generate a chimera, some of which will lead to germline transmission.

Homologous recombination using stem cells allows for the screening of gene transfer cells to identify the rare homologous recombination events. Once identified, these can be used to generate chimeras by injection of mouse blastocysts, and a proportion of the resulting mice will show germline transmission from the recombinant line. This methodology is especially useful if inactivation of the Ese1 or Ese2 gene is desired. For example, inactivation of the Ese1 or Ese1 gene can be done by designing a DNA fragment which contains sequences from a Ese1 or Ese2 exon flanking a selectable marker. Homologous recombination leads to the insertion of the marker sequences in the middle of an exon, inactivating the Ese1 or Ese2 gene. DNA analysis of individual clones can then be used to recognize the homologous recombination events.

This embodiment of the invention has the most significant commercial value as a mouse model for abnormal endocytotic activity and this may include disorders such as those involving abnormal cell division, cancer, abnormal cell migration, viral infection, abnormal tissue development and abnormal synaptic transmission disorders.

#### Therapy

Gene therapy is another potential therapeutic approach for treating disorders involving abnormal endocytosis, vesicular trafficking and abnormal regulation of the actin cytoskeleton. Such disorders may include for example but not be limited to disorders such as those involving abnormal cell division, cancer, abnormal cell migration, viral infection, abnormal tissue development and abnormal synaptic

transmission disorders.

In such therapy, normal copies of a mammalian Ese gene are introduced into patients to code successfully for normal protein in several different affected cell types. The gene must be delivered to those cells in a form in which it can be taken up and

5 code for sufficient protein to provide effective function

Retroviral vectors can be used for somatic cell gene therapy especially because of their high efficiency of infection and stable integration and expression. The targeted cells however must be able to divide and the expression of the levels or normal protein should be high. The full length Ese gene can be cloned into a  
10 retroviral vector and driven from its endogenous promoter or from the retroviral long terminal repeat or from a promoter specific for the target cell type of interest.

Other viral vectors which can be used include adeno-associated virus, vaccinia virus, bovine papilloma virus, or a herpesvirus such as Epstein-Barr virus.

Gene transfer could also be achieved using non-viral means requiring infection  
15 *in vitro*. This would include calcium phosphate, DEAE dextran, electroporation, and protoplast fusion. Liposomes may also be potentially beneficial for delivery of DNA into a cell. Although these methods are available, many of these are lower efficiency.

Antisense based strategies can employed to explore mammalian Ese gene function and as a basis for therapeutic drug design. The principle is based on the  
20 hypothesis that sequence-specific suppression of gene expression can be achieved by intracellular hybridization between mRNA and a complementary antisense species. The formation of a hybrid RNA duplex may then interfere with the processing/transport/translation and/or stability of the target Ese mRNA. Hybridization is required for the antisense effect to occur, however the efficiency of  
25 intracellular hybridization is low and therefore the consequences of such an event may not be very successful. Antisense strategies may use a variety of approaches including the use of antisense oligonucleotides, injection of antisense RNA and transfection of antisense RNA expression vectors. Antisense effects can be induced by control (sense) sequences, however, the extend of phenotypic changes are highly  
30 variable. Phenotypic effects induced by antisense effects are based on changes in criteria such as protein levels. Protein activity measurement, and target mRNA levels. Multidrug resistance is a useful model to study molecular events associated with phenotypic changes due to antisense effects, since the multidrug resistance phenotype can be established by expression of a single gene *mdr1* (MDR gene) encoding for P-  
35 glycoprotein.

Transplantation of normal genes into the affected area of the patient can also be useful therapy for any disease condition which includes abnormal endocytosis,

vesicular trafficking and abnormal regulation of the actin cytoskeleton. In this procedure, a normal human Ese gene is transferred into a cultivatable cell type, either exogenously or endogenously to the patient. These cells are then injected serotologically into the disease-affected tissue or tissues.

- 5 Immunotherapy is also possible for treating disorders which includes abnormal endocytosis, vesicular trafficking and abnormal regulation of the actin cytoskeleton. Antibodies are raised to a mutant Ese protein (or a portion thereof) and are administered to the patient to bind or block the mutant protein and prevent its deleterious effects. Simultaneously, expression of the normal protein product could  
10 be encouraged. Alternatively, antibodies are raised to specific complexes between mutant or normal Ese proteins and their binding partners.

- A further approach is to stimulate endogenous antibody production to the desired antigen. Administration could be in the form of a one time immunogenic preparation or vaccine immunization. An immunogenic composition may be prepared  
15 as injectables, as liquid solutions or emulsions. The Ese protein or other antigen may be mixed with pharmaceutically acceptable excipients compatible with the protein. Such excipients may include water, saline, dextrose, glycerol, ethanol and combinations thereof. The immunogenic composition and vaccine may further contain auxiliary substances such as emulsifying agents or adjuvants to enhance  
20 effectiveness. Immunogenic compositions and vaccines may be administered parenterally by injection subcutaneously or intramuscularly.

- The immunogenic preparations and vaccines are administered in such amount as will be therapeutically effective, protective and immunogenic. Dosage depends on the route of administration and will vary according to the size of the host.

25

#### Screening for Disease

- In another embodiment of the invention the knowledge of mammalian Ese1 and Ese2 cDNA sequences provides for screening by conventional methods to obtain the corresponding human sequences and thus screening for various diseases involving  
30 abnormal Ese1 or Ese2 (or alternative transcripts thereof) in which the defect is due to a mutant Ese1 or Ese2 gene and thus an altered and abnormal endocytosis process involved in various disorders. Mutant forms of the protein may not be able to bind with their normal binding partners and thus endocytosis, vesicular trafficking and/or actin dynamics are negatively affected. Such defects may include, for example,  
35 cancer. Other defects may include abnormal cell division, abnormal cell migration, viral infection, abnormal receptor signalling, abnormal tissue development and abnormal synaptic transmission disorders. People at a risk for such an abnormality or,

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individuals not previously known to be at risk, or people in general may be screened routinely using probes to detect the presence of a mutant Ese1 or Ese2 gene by a variety of techniques. Genomic DNA used for the diagnosis may be obtained from body cells, such as those present in the blood, tissue biopsy, surgical specimen, or autopsy material. The DNA may be isolated and used directly for detection of a specific sequence or may be PCR amplified prior to analysis. RNA or cDNA may also be used. To detect a specific DNA sequence hybridization using specific oligonucleotides, direct DNA sequencing, restriction enzyme digest, RNase protection, chemical cleavage, and ligase-mediated detection are all methods which can be utilized. Oligonucleotides specific to mutant sequences can be chemically synthesized and labelled radioactively with isotopes, or non-radioactively using biotin tags, and hybridized to individual DNA samples immobilized on membranes or other solid-supports by dot-blot or transfer from gels after electrophoresis. The presence or absence of these mutant sequences are then visualized using methods such as autoradiography, fluorometry, or colorimetric reaction. Suitable PCR primers can be generated which are useful for example in amplifying portions of the subject sequence containing identified mutations.

Direct DNA sequencing reveals sequence differences between normal and mutant Ese1 or Ese2 DNA. Cloned DNA segments may be used as probes to detect specific DNA segments. PCR can be used to enhance the sensitivity of this method. PCR is an enzymatic amplification directed by sequence-specific primers, and involves repeated cycles of heat denaturation of the DNA, annealing of the complementary primers and extension of the annealed primer with a DNA polymerase. This results in an exponential increase of the target DNA.

Other nucleotide sequence amplification techniques may be used, such as ligation-mediated PCR, anchored PCR and enzymatic amplification as would be understood by those skilled in the art.

Sequence alterations may also generate fortuitous restriction enzyme recognition sites which are revealed by the use of appropriate enzyme digestion followed by gel-blot hybridization. DNA fragments carrying the site (normal or mutant) are detected by their increase or reduction in size, or by the increase or decrease of corresponding restriction fragment numbers. Genomic DNA samples may also be amplified by PCR prior to treatment with the appropriate restriction enzyme and the fragments of different sizes are visualized under UV light in the presence of ethidium bromide after gel electrophoresis. Alternatively fluorography may be employed.

Genetic testing based on DNA sequence differences may be achieved by

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detection of alteration in electrophoretic mobility of DNA fragments in gels. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. Small deletions may also be detected as changes in the migration pattern of DNA heteroduplexes in non-denaturing gel electrophoresis. Alternatively, a single base substitution mutation may be detected based on differential primer length in PCR. The PCR products of the normal and mutant gene could be differentially detected in acrylamide gels.

Nuclease protection assays (S1 or ligase-mediated) also reveal sequence changes at specific locations. Alternatively, to confirm or detect a polymorphism restriction mapping changes ligated PCR, ASO, REF-SSCP and SSCP may be used. Both REF-SSCP and SSCP are mobility shift assays which are based upon the change in conformation due to mutations.

DNA fragments may also be visualized by methods in which the individual DNA samples are not immobilized on membranes. The probe and target sequences may be in solution or the probe sequence may be immobilized. Autoradiography, radioactive decay, spectrophotometry, and fluorometry may also be used to identify specific individual genotypes.

According to an embodiment of the invention, the portion of the DNA segment that is informative for a mutation, can be amplified using PCR. The DNA segment immediately surrounding a specific mutation acquired from peripheral blood or other tissue samples from an individual can be screened using constructed oligonucleotide primers. This region would then be amplified by PCR, the products separated by electrophoresis, and transferred to membrane. Labeled probes are then hybridized to the DNA fragments and autoradiography performed.

In a further embodiment, the invention provides pharmaceutical compositions comprising Ese1 or Ese2 proteins or a functional analogue or mimetic of these proteins or their alternative transcripts for the treatment of certain disorders characterized by abnormal endocytosis and thus cell-signalling due to lack or absence of the proteins. Such disorders may include but are not limited to abnormal cell division, cancer, viral infection, abnormal synaptic transmission as seen in central nervous disorders and abnormal cell differentiation. Such compositions as provided herein can be appropriately packaged and targeted to specific cells and/or tissues.

Administration of a therapeutically active amount of a pharmaceutical composition of the present invention means an amount effective, at dosages and for periods of time necessary to achieve the desired result. This may also vary according to factors such as the disease state, age, sex, and weight of the subject, and the ability of the Ese1 or Ese2 proteins, peptides or alternative transcripts ( for example Ese1L

and Ese2L) to elicit a desired response in the subject. Dosage regima may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

5 By pharmaceutically acceptable carrier as used herein is meant one or more compatible solid or liquid delivery systems. Some examples of pharmaceutically acceptable carriers are sugars, starches, cellulose and its derivatives, powdered tragacanth, malt, gelatin, collagen, talc, stearic acids, magnesium stearate, calcium sulfate, vegetable oils, polyols, agar, alginic acids, pyrogen-free water, isotonic saline, 10 phosphate buffer, and other suitable non-toxic substances used in pharmaceutical formulations. Other excipients such as wetting agents and lubricants, tableting agents, stabilizers, anti-oxidants and preservatives are also contemplated.

The compositions described herein can be prepared by known methods for the preparation of pharmaceutically acceptable compositions which can be administered 15 to subjects, such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable carrier. Suitable carriers and formulations adapted for particular modes of administration are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985). On this basis the compositions 20 include, albeit not exclusively, solutions of the substance in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

The pharmaceutical compositions of the invention may be administered 25 therapeutically by various routes such as by injection or by oral, nasal, buccal, rectal, vaginal, transdermal or ocular routes in a variety of formulations, as is known to those skilled in the art.

The present invention also enables the analysis of factors affecting the expression of the Ese1 or Ese2 gene in humans or in animal models. The invention further provides a system for screening candidate compounds for their ability to turn 30 on or turn off expression of the Ese1 or Ese2 gene or to screen compounds which are binding partners of these proteins.

For example, a cell culture system can be used to identify compounds which activate production of Ese1 or Ese2 proteins or, once Ese1 or Ese2 production has been activated in the cells, they can be used to identify compounds which lead to 35 suppression or switching off of Ese1 or Ese2 protein production. Compounds thus identified are useful as therapeutics in conditions where Ese1 or Ese2 production is deficient or excessive.

Compounds can also be screened in culture for their ability to overcome the effect of Ese protein expression in cell culture, tissue culture or in an animal model.

The present invention enables also a screening method for compounds of therapeutic utility as antagonists of the biological activity, binding activity, of Ese1 or  
5 Ese2 proteins and their binding partners. Those skilled in the art will be able to devise a number of possible screening methods for screening candidate compounds for Ese1 or Ese2 protein antagonism.

A screening method may also be based on binding to the Ese1 or Ese2 protein. Such competitive binding assays are well known to those skilled in the art. Once  
10 binding has been established for a particular compound, a biological activity assay is employed to determine agonist or antagonist potential.

Cell-free assays can also be readily designed by those skilled in the art to monitor and measure endocytosis, vesicular trafficking and actin dynamics.

To summarize, Ese proteins, Ese complexes including Eps15, Eps15R and  
15 many of the proteins identified in the screens as well as others identified through similar screens can be targeted for use in therapies to treat diseases including cancer, viral infection based diseases, developmental diseases due to altered cell fate specification and/or division as well as neurological diseases and diseases of altered cell migration and other diseases due to defects in the actin cytoskeleton.  
20

## **EXAMPLES**

The examples are described for the purposes of illustration and are not intended to limit the scope of the invention.

Methods of molecular genetics, protein and peptide biochemistry and  
25 immunology referred to but not explicitly described in this disclosure and examples are reported in the scientific literature and are well known to those skilled in the art.

### **Material and Methods**

#### **Ese cloning and plasmids**

30 High Stringency screening was used to isolate the two mouse Ese cDNAs by previously described methodology (58). Ese1 was cloned from an adult mouse brain cDNA library using a PCR product from nt 1707-2197 of the coding sequence as probe. Ese2 was cloned from a mixed tissue adult mouse cDNA library using a mixture of three probes EST#583881 (Research Genetics Inc.), EST#652549  
35 (Research Genetics Inc.) and a PCR product from nt 2712 to nt 3456 of the Ese2 coding sequence.



The Ese1 sequence was obtained from a single clone, whereas the Ese2 reading frame was predicted from the overlap of two cDNA clones. The DBL/PH/C2 region of Ese1L was obtained using PCR with an upstream primer designed from sequences within the DBL/PH domain region:

- 5 GAAGGAGAACTCAGACCGGCTGGAGTGGAT (this sequence was obtained from one partial Ese1L clone which we had isolated from a mouse brain cDNA library). This upstream primer was paired with downstream primers for the vector. The DBL/PH/C2 region of Ese2L was obtained using PCR with upstream and downstream primers flanking the site in Ese2 where sequence divergence had been noted within an
- 10 EST clone (upstream Ese2 sequence: GACAGAGGAGCGGTACATGGA and downstream Ese2 sequence: AGCTCCCCTGGTTCTGGCTTC). The mouse Eps15 cDNA was generated through a combination of high stringency library screening with Est sequences from the Eps15 gene and rt PCT according to established methods.

15 pcDNA3Ese1:

Full length Ese1 was cloned into the NotI site of pcDNA3 (Invitrogen Inc.). The Ese1 cDNA includes 53 nucleotides of 5' UTR plus a natural NotI site and 288 nucleotides of 3'UTR plus a small region of polylinker including a NotI site.

20 pcDNA3mycEse1:

The 5' end of pcDNA3Ese1 from the EcoRI site in the pcDNA3 polylinker to the start codon was replaced with the DNA sequence

GAATTCAGAACCATGGAACAAAAGCTTATTTCTGAAGAAGACTTGGGGCCC

- 25 ATG: where the first underline corresponds to an EcoRI site which was fused into the pcDNA3 EcoRI site and the extended underlined sequence codes for a myc-epitope tag. This is followed by nine nucleotides which code for glycine, proline and the natural Ese1 start codon. This sequence was joined to the sequences coding for amino acids 2-1213 (the remainder of Ese1). The new start codon in this tagged Ese1 construct is bolded. The 3' end of Ese1 in this vector is the same as in pcDNA3Ese1 above.

30

pcDNA3Ese1ΔC:

- The C-terminus of Ese1 was removed by replacing all sequences from nt 2209 of the coding sequence to the XhoI site in pcDNA3Ese1 with **TGACTCGAG** where the stop codon is in bold and the XhoI site is underlined. This construct codes for
- 35 amino acids 1-736 of Ese1.

pcDNA3Eps15:

This plasmid was constructed from four pieces. It contains the full length Eps15. The 5'UTR of this construct has been constructed to be GGATCCACCATG where a BamHI site is underlined and the start codon is bolded. This BamHI site was fused to the BamHI site in pcDNA3. The 3'UTR in this vector is 204 nt of the mouse natural 3'UTR fused to a short cloning linker ending in the sequence AAGCTTGGGCCC where an ApaI site is underlined; this ApaI site was fused to the ApaI site in pcDNA3.

#### pcDNA3Eps15δC:

This vector is the same as pcDNA3Eps15 except that sequences downstream from and including mouse Eps15 coding nucleotide 1500 have been replaced with CCTGGATTACAAGGATGATGATGACAAAT**G**ACTCGAG where the first underlined sequence codes for the Flag-epitope, an inframe stop codon is bolded and an XhoI site is underlined. This XhoI site was fused to the polylinker in pcDNA3. The resulting plasmid encodes amino acids 1-501 of mouse Eps15 fused to a C-terminal Flag epitope. The 5' end of Eps15 in this construct is as indicated above for pcDNA3Eps15.

#### pGBT9Ese1cc:

The Ese1 sequence coding for amino acids 330 to 732 were fused directly to GAATTC (EcoRI site) on the 5' end and to TAGGATCC (stop codon followed by a BamHI site) on the 3' end. This fragment was cloned into EcoRI/BamHI digested pGBT9 in frame with the GAL4 DBD.

#### pGBT9Ese1A3.3:

This plasmid encodes the bait for our SH3 screen. It encodes all five SH3 domains from amino acid 665-1213 and was subcloned into pGBT9 on an EcoRI fragment which fuses the Ese1 SH3 region in frame with the DBD of GAL4.

#### pGBT9Ese1Nterm:

This plasmid codes for the N-terminal 393 amino acids of Ese1, including both EH domains. It was subcloned into pGBT9 on an EcoRI/SalI fragment.

#### Yeast 2-Hybrid Screening

pGBT9Ese1cc was transformed into *S. cerevisiae* strain Y190 using 45% PEG4000, 100 mM LiAc, 10 mM Tris-HCl (pH 7.5) as per standard protocols. Cells were plated onto Sc-Trp drop-out media. Single colonies were isolated and expression

of bait was analyzed by Western Blot using antibodies against the GAL4 DBD. A clone expressing the bait fusion was used to inoculate a 100 mL Sc-Trp liquid culture grown overnight at 30°C. Cells were then re-inoculated into YPD at a density of  $5 \times 10^6$  cells/mL and grown at 30°C until the titer reached  $2 \times 10^7$  cells/mL. Cells were pelleted, resuspended in 50 mL 100 mM LiAc and incubated for 10 min at 30°C. Once again the cells were pelleted, resuspended in 20 mL PLA [35% PEG, 100 mM LiAc, 2 mg/mL salmon sperm DNA] containing 30 µg plasmid library (cloned in pAD-GAL4) incubated at 30°C for 30 min.; then heat shocked at 42°C for 40 min., pelleted, resuspended in water and plated onto [Sc-Trp-Leu-His+40 mM 3-AT]. Plates were incubated at 30°C until colonies were formed. Colonies were picked, patched and grown at 30°C overnight on Whatman filter paper laid on top of Sc-Trp-Leu-His plates. Filters were submerged in liquid nitrogen for 15 seconds then placed on top of blotting paper soaked in Z-buffer + X-gal.  $\beta$ -galactosidase activity was measured by the appearance of blue colour. Plasmids from  $\beta$ -galactosidase positive colonies were shuttled to bacteria by electroporation and isolated for sequencing.

#### Northern Blot analysis

A multiple tissue northern blot (Clontech) was prehybridized in 5 mL of ExpressHyb Solution (Clontech) at 68°C for 30 min. Probe was added at  $1 \times 10^6$  cpm/mL for 1 hr. The Blot was washed twice (2X SSC, 0.05% SDS) at room temperature, twice (0.1X SSC, 0.1% SDS) at 50°C and then exposed to film overnight.

#### Antibodies and Western Blot analysis

Western blot analysis was performed according to standard protocols. Briefly, cultured cell lines or 48 hours post transfection Cos-1 cells were washed with PBS and lysed in one ml of cold lysis buffer (50 mM Hepes pH 7.4, 150 mM NaCl, 10% glycerol, 1% Triton X100, 1 mM EGTA, 1.5 mM  $MgCl_2$ , 10 mM NaF, 10 mg  $ml^{-1}$  aprotinin, 1 mM PMSF, 10 mg  $ml^{-1}$  leupeptin, 1 mM  $Na_3VO_4$ ). Supernatants were clarified by centrifugation and immunoprecipitated with specific antiserum as indicated. Antigen-Antibody complexes were purified on anti-rabbit agarose or anti-mouse agarose (Sigma Chemical Co.) Samples were run on 7.5% PAGE gels and transferred to nitrocellulose membranes. Filters were blocked in 5% dry milk powder/0.05% Tween 20/PBS, washed in 1% dry milk powder/0.05% Tween 20/PBS, and probed with the appropriate antisera at 1mg/ml in wash buffer (In the case of chicken anti-Ese1 we used 10µg/ml to probe western blots). Probed filters were

further washed, probed again with 1/5000 dilution of horseradish peroxidase conjugated anti-rabbit IgG, anti-mouse IgG antibodies (Amersham), or anti-chicken-IgY (Zymed); washed and signal detected using the Enhanced Chemiluminescence detection system as per manufacturers instructions (Amersham).

- 5 Rabbit anti-Ese1 antisera was raised against a peptide of the following sequence: MAQFPTPFGGSLDVWAITVEE. The antisera was affinity purified over the same peptide (Research Genetics). This peptide was also used at 5µg to compete for the 5µg of antibody per immunoprecipitation reaction. Chicken anti-Ese1 antisera was raised against a fusion protein between GST and amino acids 665-1213 of mouse
- 10 Ese1. This sera was cleared of antibodies reacting against GST by incubation with glutathione s-transferase on glutathione agarose beads.

- Rabbit antibodies have also been raised against a peptide encoding the first twenty one amino acids of mouse Ese2. These antibodies were affinity purified and used to immunoprecipitate endogenous Ese1 containing protein complexes from
- 15 cultured PC12 cells. A protein of approximately 130 kDa was co-immunoprecipitated in complex with Ese2. This protein was isolated from a silver stained gel slice, digested with trypsin and the resulting peptide fragments were analyzed using both MALDI-TOF and Q-TOF Mass Spectrometers. Taken together, these mass spec analyses revealed that the Ese-2 binding protein in question is a Rho-Family exchange
- 20 protein variably known as KIAA0362, Dbs and Ost. The gene encoding KIAA0362, Dbs and Ost proteins is subject to complex alternative splicing but each protein is predicted to contain the DBL/PH exchange domain. Thus Ese proteins are found in complex with exchange proteins for the Rho-family of small GTP-binding proteins.

- 25 Monoclonal antibodies against Ese proteins were produced by immunizing mice with a GST fusion protein encoding the Ese1 C-terminus (from amino acid 665 to the stop codon) according to standard protocols. After multiple injections, the mouse spleens were removed and resuspended in phosphate buffered saline (PBS).
- 30 The spleen cells served as a source of lymphocytes. These lymphocytes were fused with a permanently growing myeloma partner cell, and the products of the fusion were plated into 96well plates in the presence of selective media. The culture supernatants were then screened by ELISA to identify those containing cells which were secreting anti-Ese antibodies. A histidine tagged version of the Ese1 C-terminus was used in
- 35 these ELISA screens to ensure that anti-Ese antibodies were detected rather than antibodies directed against the GST

portion of our original immunogen. From this procedure we isolated several independent stable hybridoma cloned lines which secrete anti-Ese antibodies. The monoclonal antibodies have then been purified on protein A/G columns as per standard protocols and demonstrated to bind Ese protein.

5

### Immunofluorescence

For Immunofluorescent staining, Cos-1 cells were typically plated at a density of  $2 \times 10^5$  per 22x50mm coverglass and transfected with 2.5 $\mu$ g of plasmid using Superfect (Qiagen Inc.). After 2 hours, the cells were washed with 10%FBS in Iscove's Media and fed with fresh 10%FBS in Iscove's Media. Two days later, these cultures were fixed with cold methanol for 30 minutes at room temperature. Cultures were washed three times 10 minutes with Phosphate buffered saline (PBS), blocked for one hour at room temperature with 1%BSA in PBS and then incubated with primary antibody in blocking solution for one hour, also at room temperature. Slides were then washed three times 10 minutes with PBS, incubated with secondary antibody/1% BSA/PBS in the dark for one hour at room temperature. Finally, slides were washed three times 10 minutes in the dark at room temperature and mounted using Dabco anti-fade solution (Sigma Chemical Co.). Slides were analyzed on the confocal microscope using the 63x objective and optical filters to separate signals on each channel.

For primary antibodies Mouse anti-myc monoclonal 9E10 (10 $\mu$ g/ml: Santa Cruz Biotech.), Rabbit anti-myc (5 $\mu$ g/ml: Upstate Biotechnology Inc.), Rabbit anti-Eps15 antisera #C20 (1 $\mu$ g/ml: Santa Cruz Biotech), Rabbit anti-Flag epitope antisera (5 $\mu$ g/ml: Zymed) and mouse anti-Dynamin I #D25520, which recognizes Dynamin in Cos-1 cells by both immunoprecipitation and western blotting (data not shown) (20 $\mu$ g/ml: Transduction labs, Inc.) were used. As secondary antibodies we used FITC-labelled goat anti-mouse (1:80 dilution) and Texas Red-labelled goat anti-rabbit antibodies (1:100 dilution) (Jackson Immuno Research Laboratories Inc.). Endogenous Eps15 staining above background in non-transfected cells was undetected. Prominent cytoplasmic Eps15 staining in transfected cells obscured the detection of clathrin-coated pit associated Eps15 at the membrane (Figure 4B). In Figure 5B panel a and b, Dynamin staining with both primary and secondary antibodies was performed first. MycEse1 was then stained for using biotinylated 9E10 followed by streptavidin conjugated to Texas Red. Following application of the secondary antibody to stain for Dynamin, all further incubations and washes were

performed under dark condition. Endocytosis assays were performed as previously described (6, 9).

Mouse Ese1 cDNA - Sequence ID NO:1

5 CGGCACGAGGAGGAGTGGAGCGGCGCGGGAGGGCGCGCAGCTTGGTTGC  
TCCGTAGTACGGCGGCTCGCAAGGGAGCATCCCGAGCGGGCTCCGGGACG  
GCCGGGAGGCAGGCAGGCGGGCGGGCGGGGATGGTGTGCGCGGCTGCGG  
ACTCGGCGTTCCTCGCGCGGCGTGCGGGCTGCACTGATTTGTGTGAGGGG  
CGGCCGCGCGCACCCGCCCGGAGATGAGGCGTCGATCAGCAAGGTGAAC  
10 GTAATAGAACCATGGCTCAGTTTCCCACACCTTTCGGTGGTAGCCTGGATG  
TCTGGGCCATAACTGTGGAGGAAAGGGCCAAGCATGACCAGCAGTTCCTT  
AGCCTGAAGCCGATAGCGGGATTTATTACTGGTGATCAAGCGAGGAACTT  
TTTTTTCCAATCTGGGTTACCTCAGCCTGTCTTAGCACAAATATGGGCGCT  
AGCGGACATGAATAACGATGGAAGGATGGATCAAGTGAATTTTCCATAG  
15 CCATGAAGCTTATCAAACCTGAAGCTACAAGGATATCAGCTCCCCTCCACA  
CTTCCCCCTGTCATGAAACAGCAACCAGTGGCTATTTCCAGTGCACCAGCA  
TTTGGTATAGGAGGGATTGCTAGCATGCCACCACTCACAGCTGTTGCTCCT  
GTGCCAATGGGCTCCATTCCAGTTGTTGGAATGTCTCCACCCTTAGTATCT  
TCTGTCCCTCCAGCAGCAGTGCTTCCCCTGGCTAACGGGGCTCCTCCCGTC  
20 ATACAGCCTCTGCCTGCGTTTGCGCATCCTGCAGCCACATGGCCAAAGAG  
TTCTTCCTTCAGCAGATCTGGTCCAGGGTCACAATTAAACACTAAGTTACA  
GAAGGCACAATCATTGATGTCGCCAGCGCCCCTCCAGCAGCAGAATGGG  
CTGTGCCTCAGTCATCAAGGCTGAAATACAGGCAGTTATTCAACAGCCAC  
GACAAAACCTATGAGTGGACACTTAACAGGTCCCCAGGCAAGAACTATTCT  
25 CATGCAATCAAGTTTACCCAGGCTCAGCTGGCTTCAATATGGAATCTTTC  
TGACATTGATCAAGATGGAAAACCTCACTGCAGAAGAATTTATCCTAGCTA  
TGCACCTAATTGATGTTGCCATGTCTGGTCAGCCACTGCCGCCCCGTCCTGC  
CTCCAGAATACATCCCTCCTTCCTTCAGAAGAGTTCGCTCCGGCAGTGGGA  
TGTCCGTCATAAGCTCTTCTTCTGTGGATCAGAGGCTGCCTGAGGAGCCGT  
30 CGTCAGAGGATGAGCAGCAGCCAGAGAAGAACTGCCTGTGACATTTGAA  
GATAAGAAGCGGGAGAACTTCGAGCGAGGCAGTGTGGAGCTGGAGAAGC  
GCCGCCAAGCGCTCTTGGAGCAGCAGCGCAAAGAGCAGGAGCGGTTGGC  
TCAGCTGGAGCGCGCCGAGCAGGAGAGGAAAGAGCGGGAGCGCCAGGAG  
CAGGAGGCCAAGCGGCAGCTGGAGCTGGAGAAGCAGCTGGAGAAGCAGC  
35 GGGAGCTGGAGCGGCAGCGAGAGGAGGAGGAGGAAGGAGATCGAGA  
GGCGCGAGGCCGCAAAACGGGAACTGGAAAGGCAGCGACAACCTGAATG  
GGAACGGAACCGGAGACAGGAACTCCTGAATCAGAGGAACAAGGAGCAG

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GAGGGCACCGTGGTCCTGAAGGCAAGGAGGAAGACTCTGGAGTTTGAGTT  
AGAAGCTCTGAATGACAAAAAGCATCAGCTAGAAGGAAAACCTTCAGGAT  
ATCAGGTGTCGACTGGCAACCCAGAGGCAAGAAATTGAGAGCACGAACA  
AGTCTAGAGAGCTAAGAATTGCTGAAATCACCCACTTACAGCAGCAGTTG  
5 CAGGAATCTCAGCAAATGCTTGGAAGACTTATTCCAGAGAAACAGATACT  
CAGTGACCAGTTAAAACAAGTCCAGCAGAACAGTTTGCATAGAGACTCGC  
TTCTTACCCTCAAAAGAGCCTTGGAAGCAAAGGAGCTGGCCCCGGCAGCAG  
CTCCGGGAGCAGCTGGACGAGGTGGAGAGAGAGACCAGGTCAAAGCTGC  
AGGAGATTGATGTTTTCAACAACCAGCTGAAGGAACTGAGAGAGATACAT  
10 AGCAAACAGCAACTCCAGAAGCAGAGGTCCCTGGAGGCAGCGCGACTGA  
AGCAGAAAGAGCAGGAGAGGAAGAGCCTGGAGTTAGAGAAGCAAAAGG  
AAGACGCTCAGAGACGAGTTCAGGAAAGGGACAAGCAATGGCTGGAGCA  
TGTGCAGCAGGAGGAGCAGCCACGCCCCGGAAACCCACGAGGAGGAC  
AGACTGAAGAGGGAAGACAGTGTGAGGAAGAAGGAGGCGGAAGAGAGA  
15 GCCAAGCCGGAAATGCAAGACAAGCAGAGTCGGCTTTTCCATCCGCATCA  
GGAGCCAGCTAAGCTGGCCACCCAGGCACCCTGGTCTACCACAGAGAAAG  
GCCCCGCTTACCATTTCTGCACAGGAGAGTGTAAGAGTGGTATATTACCGA  
GCGCTGTACCCCTTTGAATCCAGAAGTCACGATGAGATCACCATCCAGCC  
AGGAGATATAGTCATGGTGGATGAAAGCCAGACTGGAGAGCCAGGATGG  
20 CTTGGAGGAGAGCTGAAAGGGAAGACGGGATGGTTCCCTGCAAACATATGC  
AGAAAAGATTCCAGAAAATGAGGTTCCCACTCCAGCCAAACCAGTGACCG  
ATCTGACATCTGCCCCTGCCCCCAAACCTGGCTCTGCGTGAGACCCCTGCTC  
CTTTGCCAGTGACCTCTTCTGAGCCCTCCACAACCCCCAACAACCTGGGCAG  
ACTTCAGTTCCACGTGGCCCAGCAGCTCAAACGAGAAGCCAGAAACGGAC  
25 AACTGGGATACGTGGGCGGCTCAGCCTTCTCTGACCGTACCTAGTGCTGG  
CCAGTTACGGCAGAGATCAGCCTTTACCCAGCCACAGCCACTGGCTCCT  
CCCCATCTCCCGTCCTGGGCCAGGGTGAAAAGGTGGAAGGGCTACAAGCG  
CAAGCCCTGTATCCCTGGAGAGCCAAAAAAGACAACCACTTAAATTTTAA  
CAAAAGTGACGTCATCACCGTTCTGGAACAGCAAGACATGTGGTGGTTTG  
30 GAGAAGTTCAAGGTCAGAAGGGTTGGTTCCCCAAGTCTTACGTGAAACTC  
ATTTAGAGGGCCCGTAAGGAAATCCACAAGCATCGATACTGGCCCTACTGA  
AAGTCCTGCTAGTCTAAAGAGAGTGGCTTCCCCGGCCGCAAGCCAGCCA  
TTCCCGGAGAAGAGTTTATTGCCATGTACACATACGAGAGTTCTGAGCAA  
GGAGATTTAACCTTTCAGCAAGGGGATGTGATTGTGGTTACCAAGAAAGA  
35 TGGTGAAGTGGTGGACGGGAACGGTGGGCGACAAGTCCGGAGTCTTCCCTT  
CTAACTATGTGAGGCTTAAAGATTCAGAGGGCTCTGGAAGTGTGGGAAA  
ACAGGGAGTTTAGGAAAAAAACCTGAAATTGCCAGGTTATTGCTTCCTA

CGCTGCTACTGGTCCCGAACAACCTCACCTGGCTCCTGGGCAGCTGATTCT  
 GATCCGGAAAAAGAACCCAGGTGGATGGTGGGAAGGAGAACTGCAAGCT  
 CGAGGGAAAAAGCGCCAGATAGGGTGGTTTCCAGCAAATTATGTCAAACCT  
 TCTAAGCCCCGGAACAAGCAAAATCACCCCAACTGAGCTACCCAAGACCG  
 5 CAGTGCAGCCAGCAGTGTGCCAGGTGATCGGGATGTACGATTACACCGCC  
 CAGAACGATGACGAACTAGCCTTCAGCAAAGGCCAGATCATCAACGTCCT  
 CAACAAGGAGGACCCGGACTGGTGGAAAGGAGAAGTCAGTGGGCAAGTT  
 GGGCTCTTCCCATCCAATTATGTAAAGCTGACCACAGACATGGACCCCGAG  
 CCAGCAATGAATCATATGTTGTCCATCCCCCCTCAGGCTTGAAAGTCCTC  
 10 AAAGAGACCCACTATCCCATATCACTGCCCAGAGGGATGATGGGAGATGC  
 AGCCTTGATCATGTGACTTGCAGCATGATCACCTACTGCCTTCTGAGTAGA  
 AGAACTCACTGCAGAGCAGTTTACCTCATTTGACCTTAGTTGCATGTGATC  
 GAAATGTCTGAGTCACTGCGTGCAGAGGCAGAAGCAAATTGCAGAACTGC  
 ACAGGGTGGTGGGTCCTTTTGGGGCTTTCCTAGTCACTCAGACTGACCGGC  
 15 CCCGCCTTCACACGGGCGCTTTCAATAGTTTTAAGATTATTTTTAAATGTG  
 TATTTTAGCCTTTTAATAAAAAATCTCAATCAATTACTTCTTTGCCTATTTTG  
 GTTTTACAAAAACACCCACTATCAAGGAGTGCCTGTCTGCGGACGATTAA  
 AATGCTGTTCCGGGCGTACCGTAAACTGAGAGCTTGCTGTACCTTTGCCGT  
 TTGTCCAGTGTTCCTCAACCACATTGTGTAGTTTGGGGCTGTTCCCTGCCGT  
 20 AGAGCACAGAGGAGATGGGTGTACCTGTTTTGAAAATGTGTATGTAGACT  
 GAGCCTGACTATGGAAGGGGTTATGCTTGTCTGTGACCATCACGTGTACCT  
 GTCGCGCATGTACCATCTGTACCGAAGAAGTAGCTCTTCCTCCATGGCTAA  
 ACCCACCACCGTGTACAGTGCTCTCATCTACTGCATTCAATTTTACTTTGCA  
 CAGTGACCTTGTAGCCACCTGAGGAAGCACCCATGTTTCCGTTTGGTCTCA  
 25 GATGTACCTAGTTGTGCCCCGTGTTTTGTTTTTATTTTTCAATCTGGCATGTC  
 TTCACACCATAAACTAGTAAGACGCCAACTGCCAGGCGGTTACGATCAT  
 CAGTACCCACCGTCTTAGTCTCTGTACGTGAAGTTTATTCCAGTTGCTTTT  
 TATGGAATATCTTGAACAAGTAATCTTCTTGACAAGAAAGAATGTATAGA  
 AGTCTCCCTGCAATTAATTTCCAGTGTTTACATTTTTTAACTAGACTGTGG  
 30 GGGTTGCTACAGATTAATATGAAATGGCGCTCCTGGTCCGTGTGTGTGTTA  
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 GAAGAGAATTCGATATCAAGCTT

Mouse Esi1 coding sequence - Sequence ID NO:2

35 ATGGCTCAGTTTCCCACACCTTTCGGTGGTAGCCTGGATGTCTGGGCCATA  
 ACTGTGGAGGAAAGGGCCAAGCATGACCAGCAGTTCCTTAGCCTGAAGCC  
 GATAGCGGGATTTATTACTGGTGATCAAGCGAGGAACTTTTTTTTCCAATC



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TGGGTTACCTCAGCCTGTCTTAGCACAAATATGGGCGCTAGCGGACATGA  
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ATCAAACCTGAAGCTACAAGGATATCAGCTCCCCTCCACACTTCCCCCTGTC  
ATGAAACAGCAACCAGTGGCTATTTCCAGTGCACCAGCATTTGGTATAGG  
5 AGGGATTGCTAGCATGCCACCACTCACAGCTGTTGCTCCTGTGCCAATGG  
GCTCCATTCCAGTTGTTGGAATGTCTCCACCCTTAGTATCTTCTGTCCCTCC  
AGCAGCAGTGCCTCCCCTGGCTAACGGGGCTCCTCCCGTCATACAGCCTCT  
GCCTGCGTTTGCGCATCCTGCAGCCACATGGCCAAAGAGTTCTTCCTTCAG  
CAGATCTGGTCCAGGGTCACAATTAAACACTAAGTTACAGAAGGCACAAT  
10 CATTCGATGTGCGCCAGCGCCCCTCCAGCAGCAGAATGGGCTGTGCCTCAG  
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15 GATGTTGCCATGTCTGGTCAGCCACTGCCGCCCGTCCTGCCTCCAGAATAC  
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25 GTTTAGGAAAAAAACCTGAAATTGCCCAGGTTATTGCTTCCTACGCTGCTA  
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35 CCACTATCCCATATCACTGCCAGAGGGATGA

Mouse Esl protein - Sequence ID NO:3

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 5 ANGAPPVIQPLPAFAHPAATWPKSSSFSSRSGPGSQLNTKLQKAQSFVVASAPP  
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 SVISSSSVDQRLPEEPSEDEQQPEKKLPVTFEDKKRENFERGSVELEKRRQAL  
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 10 EERRKEIERREAAKRELERQRQLEWERNRRQELLNQRNKEQEGTVVLKARR  
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 QLQESQQMLGRLIPEKQILSDQLKQVQQNSLHRDSSLTLKRALEAKELARQQ  
 LREQLDEVERETRSLQLEIDVFNNQLKELREIHSKQQLOKQRSLEAARLKQKE  
 QERKSLELEKQKEDAQRREVQERDKQWLEHVQQEEQPRPRKPHEEDRLKRED  
 15 SVRKKEAEERAKPEMQDKQSRLFHPHQEPAKLATQAPWSTTEKGPLTISAQE  
 SVKVYYRALYPFESRSHDEITIQPGDIVMVDESQTGEPGWLGGELKGKTGW  
 FPNYAEEKIPENEVPTPAKPVTDLTSAPAPKLALRETPAPLPVTSSEPSTTPNN  
 WADFSSTWPSSSNEKPETDNWDTWAAQPSLTVPSAGQLRQRSAPATATGS  
 SPSPVLGQGEKVEGLQAQALYPWRAKKDNHLNFNKSDVITVLEQQDMWWF  
 20 GEVQQGQGWFPKSYVKLISGPVRKSTSIDTGPTESPASLKRVASPAKPAIPGE  
 EFIAMTYESSEQGDLTQQGDVIVVTKKGDGWWTGTGVDKSGVFPSNYVR  
 LKDSESGTAGKTGSLGKKPEIAQVIASAAATGPEQLTLAPGQLILIRKKNPBG  
 WEGELQARGKKRQIGWFPANYVKLLSPGTSKITPTLPAVQPAVCQVIG  
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 25 DMDPSQQ

Mouse Ese2 cDNA - Sequence ID NO:4

CCCTTCCTTTCTTTTTTTGTGTTTCGCTTCGGCCGTGCCGGCTGAGAGCCC  
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 30 TGACCCGGCGCGCGCGCGCGCGCACGGCAGGGTCTTCCCGGAGCTTGGC  
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 GCGCGAGGCGCCAGATGGCTGAGAGCTGCAAGAAGAAGTCAGGATCATG  
 ATGGCTCAGTTTCCACAGCGATGAATGGAGGGCCAAATATGTGGGCTAT  
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 35 CTTAGGAGGTTACATAACAGGTGATCAAGCCCGTACTTTTTCTACAGT  
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ATGAAACAACCCCCCTATGTTCTCTCCACTAATCTCTGCTCGTTTTGGGATG  
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5 ATGATGCCTGCTCCCCTAGTGCCTTCTGTTAGTACATCCTCATTACCAAAT  
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CCTCAACTGCTTCCCTCTCAGGGAAGTACCTAAGACAGGGACCTCAGAG  
10 TGGGCAGTTCCCTCAGCCTTCAAGATTAAAGTATCGGCAAAAATTTAATAGT  
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15 TTGCCTCCCGAGCTTGTCCCTCCATCTTTCAGAGGGGGAAAGCAAGTTGAT  
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30 AGCTCAGTAACACACCTGATTCAGGGATCAGTTTACTTCATAAAAAGTCA  
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15 AGTTACCTCCACAGCCTATCCAGTTGGAGAAGACTACATTGCACTTTATTC  
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20 GCTCAAGTAACTTCAGCATATGCTGCTTCAGGGACTGAGCAGCTCAGCCTT  
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25 TAACGAAGATGAGCTCAATTTCTCAAAGGACAGCTGATTAATGTTATGA  
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30 ATGGACGACCTGCAACTTTTTGAACAAAAAACTCTCCTTTGAGGGCCTGG  
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35 GTACTAGTGTTAACCTGTTTACAGCTGTGTTAGATGCCAGGGTTTCCATTTTC  
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CACACTATGTGACCTCTGCTGGGTACACACTCAGGAGGTGAAGGGCTGA  
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5 ATAAGGAGGAAAGCTTGATGTACTTTAAATATACTGTGAACTCTAATAAT  
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AAAAAAAAAAAAAAAAA

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Mouse Ese2 coding sequence - Sequence ID NO:5

ATGGCTCAGTTTCCCACAGCGATGAATGGAGGGCCAAATATGTGGGCTAT  
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15 CAGGTCTGCCGGCCCCGGTTTTAGCTGAAATATGGGCCTTATCAGATCTGA  
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20 ATAGCAACACCCTTGTCTTCTGCTACGTCAGGGACCAGTATTCCTCCCCTA  
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25 CCTCAACTGCTTCCCTCTCAGGGAACCTACCTAAGACAGGGACCTCAGAG  
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30 CGATGCACCTCACTGACATGGCCAAAGCTGGACAGCCACTACCACTGACG  
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35 GCAGCAGAGGGAGGCTGAACGCAAAGCCCAGAAAGAGAAGGAAGAGTG  
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5 CAGCAGATCTCAGGCAGACTACAAGATGTCCAAATCAGAAAGCAAACAC  
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10 TCAGAAAAGGAAGAATTATGCCAAAGACTTAAAGAACAATTAGATGCTCT  
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15 AGCAAAGCAAGGAAAAGAAAAGTGTGGAGAGAAAGTATTAGAAAGGAA  
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 5 ACAAAGATGACCCTGACTGGTGGCAAGGAGAAACCAATGGTCTGACTGGT  
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 CAGTGGTGTGCTGACCTCCAAGCCCTGGACACAATGCAGCCTACGGAGAG  
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 ATGGACGACCTGCAACTTTTTGAACAAAAAACTCTCCTTTGA

10

mouse Ese2 - Sequence ID NO:6

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 15 VPSVSTSSLPNGTASLIQPLSIPYSSSTLPHASSYSLMMGGFGGASIQKAQSLID  
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 QARNALLQSNLSQTQLATIWTADIDGQQLKAEFILAMHLTDMAGAGQPL  
 PLTLPELVPPSFRGGKQVDSVNGTLPSYQKTQEEEPQKKLPVTFEDKRKANY  
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 20 LEKRLEKQRELERQREEERRKEIERREAAKQELERQRRLEWERLRRQELLSQK  
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 HKKSSEKEELCQRLKEQLDALEKETASKLSEMDSFNNQLKELRESYNTQQLA  
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 25 EKQKRLQEEKSQDKTQEEERKAEAKQSETASALVNYRALYPFEARNHDEMS  
 FSSGDIIQVDEKTVGEPGWL YGSFQGKFGWPCNYVEKVLSSEKALSPKKALL  
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 30 IALYSYSSVEPGDLTFTEGEEILVTQKDGEWWTGSIGERTGIFPSNYVRPKDQE  
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35

Mouse Ese2 alternative transcript partial cDNA sequence - Sequence ID NO:7



-49-

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 CGTTCTCAAAAGACTTCAGGTATTGGGCGTCTGATGGTGCATGTCATTGAA  
 5 GCTACAGAATTAAAAGCCTGCAAACCAAACGGGAAAAGTAATCCATACTG  
 TGAAGTCAGCATGGGCTCCCAAAGCTATACCACCAGGACCCTGCAGGACA  
 CACTAAACCCCAAGTGGAACTTCAACTGCCAGTTCTTCATCAAGGATCTTT  
 ACCAGGACGTTCTGTGTCTCACTATGTTTGACAGAGACCAGTTTTCTCCAG  
 ATGACTTCTTGGGTCGTACTGAAGTTCCAGTGGCAAAAATCCGAACAGAA  
 10 CAGGAAAGCAAAGGCCCCACCACCCGCCGACTACTACTGCACGAAGTCCC  
 CACTGGAGAAGTCTGGGTCCGCTTTGACCTGCAACTTTTTGAACAAAAAA  
 CTCTCCTTTGAGGGCCTGGGGAAGCCAGAACCAGGGGAGCTGCCCCAAG  
 GCTGGGTCTAAAGACAGATTTTGCTCTCCAGGACAGAGGAGCATCACAT  
 GGCTTCATCCATCAAACAGCCACACTCGCTGGGCCTGTATTTTATTGCACA  
 15 CTAAATTGCTAGCAATCTATGCAAACATGATCTTT

Mouse Ese2 alternative partial protein containing C2 membrane-binding domains -  
 Sequence ID NO:8

VFHISHIDRVYTLRTDNINERTAWVQKIKGASEQYIDTEKKKREKAYQARSQK  
 20 TSGIGRLMVHVIEATELKACKPNGKSNPYCEVSMGSQSYTTRTLQDTLNPKW  
 NFNCQFFIKDL YQDVLCLTMFDRDQFSPDDFLGRTEVPVAKIRTEQESKGPTT  
 RLLLLHEVPTGEVWVRFDLQLFEQKTL

**Novel Ese-coiled-coil interacting clones:**

25 Mouse homologue of C07E3.1 protein (clone 65): - Sequence ID NO:9

GAATTCGGCACGAGGGCTGAGAGAAGCGGACTCCGAGGACTCTGATGCTG  
 AAGAGAAGCCTGTAAAGCAGGAGGACTTCCCGAAGATTTAGGACCAAAG  
 AAGTTAAAGACGGGTGGCAATTTTAAGCCCAGCCAGAAAGGCTTTTCAGG  
 AGGAACCAAGTCCTTCATGGACTTTGGCAGCTGGGAGAGACACACGAAAG  
 30 GGATCGGGCAGAAGCTGCTGCAGAAGATGGGCTACGTCCCTGGGCGTGGC  
 CTGGGGAAGAACGCACAGGGGATCATCAACCCCATCGAAGCCAAACAGA  
 GAAAAGGCAAGGGAGCCGTGGGGGCCTATGGCTCGGAGAGGACCACTCA  
 GTCTCTGCAGGACTTCCCCGTGGCCGACTCGGAAGAGGAGGCAGAAGAGG  
 AGTTTCAGAAGGAGCTGAGCCAATGGAGGAAAGACCCAGCGGGAGCAA  
 35 GAAGAAGCCAAAGTACTCTTACAAGACTGTGGAGGAGCTGAAGGCCAAG  
 GGCAGGGTCAGCAAGAAGCTCACAGCACCTCAGAAGGAACTGTCTCAGGT  
 CAAGGTGATCGACATGACAGGCCGGGAGCAGAAGGTGTACTACAGCTAC

AGCCAAATCAGCCACAAGCACAGCGTGCCCGATGAAGGGGTGCCATTGCT  
 GGCGCAGCTGCCCCCACAGCCGGCAAGGAAGCCAGGATGCCGGGCTTTG  
 CACTGCCTGAGCTGGAGCACAACTGCAGCTGCTCATTGAGCGCACGGAG  
 CAGGAGATCATCCAGAGCGACCGGCAGCTCCAGTATGAGCGGGACATGGT  
 5 GGTCAGCCTGTCGCATGAGCTGGAGAAGACGGCCGAGGTTCTTGACATG  
 AGGAGCGTGTCATCTCTAACCTCAGCAAGGTGCTGGCCCTGGTGGAGGAA  
 TGTGAGCGCCGCATGCAGCCCCATGGCACCGACCCCTCACTCTGGATGA  
 GTGTGCCCCGCATCTTTGAGACACTACAGGACAAGTATTATGAGGAGTACC  
 GCCTGGCGGACCGCGCAGACCTCGCTGTGGCCATTGTCTACCCGCTCGTG  
 10 AAGGACTACTTTAAGGATTGGCACCCCTCGAGGG

Mouse partial C07E3.1 protein (clone 65): - Sequence ID NO:10

GTKSFMDFGSWERHTKGIGQKLLQKMGYVPGRGLGKNAQGIINPIEAKQRKG  
 KGAVGAYGSERTTQSLQDFPVADSEEEAEFEQKELSQRKDPSSGSKKKPKY  
 15 SYKTVEELKAKGRVSKKLTAPQKELSQVKVIDMTGREQKVYYSYSQISHKHS  
 VPDEGVPLLAQLPPTAGKEARMPGFALPELEHNLQLLIERTEQEIIQSDRQLQY  
 ERDMVVSLSHELEKTAEVLAHEERVISNLSKVLALVEECERRMQPHGTDPLT  
 LDECARIFETLQDKYYEYRLADRADLAVAIVYPLVKDYFKDWHPSR

20 Mouse Novel cDNA clone 42/ Est accession #W29719 and #AA915044: - Sequence ID NO:11

CATGGCGGCGGCTGCGGAGGGCGTCCCGGCGACGCGACGGAGGACGAGC  
 CACCTCGAGATGATGCTGCGGTGGAGACAGCCGAGGAAGCAAAGGAGC

25 Mouse Novel cDNA clone 70: - Sequence ID NO:12

CTTGAGTCTACTGAAAATACCCTGCAGGAAGCTACATCATCCATGTCTTTG  
 ATGACCCAATTTGAACAGGAAGTATCTGGCCTCCAAAGACCATACGTGAT  
 ATTGAGACTAGCGAAGAGATGC

30 Mouse Novel cDNA clone 83/Est accession #AA589041 and W98708: - Sequence ID NO:13

GAATTCGGCACGAGGGAGTCTGGTTCTGGAAAGCCGACAGAAGCTGAGCT  
 TGTCAACTTAGATTTCTTGGGAGATTGGATGTTCCGGTATCTGCCCCACC  
 CCTGTGTGTCTGAGCTCGAGTCTCTGCTGGACTATGG  
 35

**Novel Ese-SH3 interacting clones:**

Mouse YNK1 partial cDNA: - Sequence ID NO:14

CTTTACGAGCAGAGGGAGCCAAATTCAGAGCCGTTTTAGATAAAGCTGTG  
CAAGCGGATGGACAGGTGAAGGAGCGCTACCAGTCCCATCGAGACACCA  
TCGCACTTCTGTGTAAGCCGGAGCCAGAGCTGAATGCTGCCATCCCCTCTG  
CTAACCCAGCAAAGACCATGCAGGGCAGCGAGGTTGTAAGTGTCTTAAAG  
5 TCCTTATTATCAAATCTTGATGAAATCAAGAAGGAAAGAGAGAGTCTTGA  
GAATGACCTGAAGTCAGTGAATTTTGACATGACAAGCAAGTTTTTGACAG  
CTCTGGCCCAAGATGGCGTGATAAATGAGGAGGCTCTCTCTGTCACTGAG  
CTGGATCGGATCTATGGCGGTCTAACAAGTAAAGTTCAAGAGTCTCTGAA  
GAAACAAGAGGGACTTCTAAAAAATATACAGGTCTCACACCAAGAATTCT  
10 CCAAAATGAAGCAATCTAACAACGAGGCTAACTTGAGAGAAGAAGTTCTG  
AAGAACCTAGCAACTGCGTATGACAACCTTTGTTGAGCTTGTAGCTAACTTG  
AAGGAGGGCACAAGTTTTACAATGAGCTGACTGAGATCCTGGTCAGGTT  
CCAGAACAAATGCAGTGACATAGTGTTCACGGAAGACAGAAAGAGAC  
GAGCTCTTGAAGGATCTGCAGCAGAGCATTGCCAGAGAGCCCAGCGCTCC  
15 TTCAATCCCTCCTCCAGCCTATCAGTCCTCCCAGCAGCGGGGCATGCAGC  
AGCGCTCCAACCTCCAGCCCCAAGAACCATGCCGCCTGCTAAGCCCCAGC  
CTCCAGCCCCGGCCTCCACCTCCTGTGCTTCCTGCAAACCGAGTTCCTCCTG  
CTTCTGCTGCTGCTGCCCTGCAGGCGTGGGGACGGCTTCAGCAGCGCCG  
CCACAGACCCCTGGCTCTGCTCCCCCGCCACAGGCTCAGGGACCACCATA  
20 CCCTACCTATCCAGGATATCCCGGGTATTGCCAAATGCCCATGCCCATGGG  
CTACAACCCCTACGCATATGGCCAGTACAATATGCCGTACCCACCGGTGT  
ATCACCAGAGCCCCGGACAGGCTCCATACCCAGGACCCCAGCAGCCTACC  
TACCCCTTCCCTCAGCCCCCGCAGCAGTCCTACTATCCACAGCAGTAACGC  
TGCCACGTGCTGCTGGTTCAGATCAGAGCGACAGGACAGCAGCTGCCACC  
25 AGCTCTAAGCCACGCTCTGGCCACTCGAGAGTATCTTGCTCTATTGATTGC  
TGTGGATGATTTCTGTCTGTGGCTAAAGCCGAAGGCTGGGCCCCACCTCCA  
CATTTGATCGCACTCGTGAGATTCTGCTGCTGTTGCAGTATAAACGCTAGC  
TATAATAGCATTTGAAAAAAATTACAGTTCCATAAAATGCTGAAAATGAG  
AAATTAAACCTGCAAGTGAAACATTTGAAATTAGCATACTTTATAAGATG  
30 CAGTTGGGACAAAGATGGCTTAAGTACTGATATTTAAGGAAAAAGTTTTCT  
TTTCTCTTTTGGTTTATTGATTTAGTTTAATTTCTATTATGATATTTTGCATA  
ATCAAGGCATTGTAAATCTTATAATTTAAAAATAAATTACTTACGAACAGT  
TGTCATTGTTATGTTTTGTCATTGATTCTCATTGCTGTCTAGTTCCTTTCTG  
GTATTAGCCTCTCCTTCTGTATGTTACAGGCTCCATTACTGTGTTGAATTG  
35 CGTGACGTCAGGTGAGCAGTCAGGGAGGGCTGCTCTGCGGACGCCAAGCG  
CACACCAGCTTGTCTCAGGCTCAGCAGTCAGCTCATCTGGACATTTCTATT  
TAAAAGTCCTTTAATGTGGAAGATACACACAATTGTTACCAAAGGTTCTTC

CAATTAATTTTACAATTTAAAAAGTATGTATTAATGTTTTATTGTTAGATTT  
 TCCAAAAAATGATGCAAATTCTGGTAATATTCATTTCCCTCACCCATAAT  
 TTGGTTAAAATGAGTAGTTTTAGCCATACAGTCTCATCTGCTGTGGAGGAA  
 CCTGGAGAAAGTCCCCTGTGCCTTTCTAGCCCTTGGGTTCTATTCTTATCCT  
 5 GCAATGTCTACTGCACAGTGTGTTTGAGCAGATCCTAACCCTCCTTTTACA  
 GTTTCTTCTTCTTACTTCTTTATTCTTTTTGTGGCTCCTGAAATCTGAGGTTA  
 TTTTGTAAATTCAGGAGCATGCAGGACAATTGTTGGGACATGTGCCTAGTCC  
 GGAATACAGCCCAGGACAGCAAGGAGATGCGTCCTGCACCAGGAAGCCG  
 TGCAGGCAGGAGCTGTCCAAGGTCCCGGCGGCTCTGCCTGTGTGAGGCAG  
 10 GAGAATGAGCAGATTCCCTAATCTATGTTCTCGAAGTTTAATGCTGATGTT  
 GTCTTGCCTTATCCTCATTTAACTGATACTGTCACCCAGTCCACCTTTGCTC  
 TCATTGCAAAGTGATAGTGTAATTTCAAATGTAAGACTGAAGATACGATT  
 GTAAAAGGGAGTAACTGGTTTAAACGTGTTATTCTAAAGCACCTTACTTT  
 GTTGTGTTGATGCAGAAAACACAGATGCGCTAATTCAGTATAAATGACTGA  
 15 TTGCCTGGAATTTGGACGTTGGCTTAAAGTCCGATAGCTAAACCTTGGCAA  
 AACATAACAAACATTTTCATTGCTCAGCCTCAGTGCTCTGGAGTATTCAGTG  
 TATGAGACAGGTTTATTTGAGTCCTCTGTAAATGGCATTGGAATTTTATAT  
 TCTCCCTCCCGAGTATCTTATAAGACATCCCCTGAGTTAGGGAGTTCCCA  
 GACTGCTACTCTATTCTTATGAATGCAAAACAACCACCAATAGAACAAA  
 20 AAAAAAAAAAAAAAACTCGAG

Mouse YNK1 partial coding cDNA - Sequence ID NO:15

CTTTACGAGCAGAGGGAGCCAAATTCAGAGCCGTTTTAGATAAAGCTGTG  
 CAAGCGGATGGACAGGTGAAGGAGCGCTACCAGTCCCATCGAGACACCA  
 25 TCGCACTTCTGTGTAAGCCGGAGCCAGAGCTGAATGCTGCCATCCCCTCTG  
 CTAACCCAGCAAAGACCATGCAGGGCAGCGAGGTTGTAAGTGTCTTAAAG  
 TCCTTATTATCAAATCTTGATGAAATCAAGAAGGAAAGAGAGAGTCTTGA  
 GAATGACCTGAAGTCAGTGAATTTTGACATGACAAGCAAGTTTTTGACAG  
 CTCTGGCCCAAGATGGCGTGATAAATGAGGAGGCTCTCTGTCACTGAG  
 30 CTGGATCGGATCTATGGCGGTCTAACAAGTAAAGTTCAAGAGTCTCTGAA  
 GAAACAAGAGGGACTTCTAAAAAATATACAGGTCTCACACCAAGAATTCT  
 CCAAAATGAAGCAATCTAACAACGAGGCTAACTTGAGAGAAGAAGTTCTG  
 AAGAACCTAGCAACTGCGTATGACAACCTTTGTTGAGCTTGTAGCTAACTTG  
 AAGGAGGGCACAAAGTTTTACAATGAGCTGACTGAGATCCTGGTCAGGTT  
 35 CCAGAACAAATGCAGTGACATAGTGTTTGCACGGAAGACAGAAAGAGAC  
 GAGCTCTTGAAGGATCTGCAGCAGAGCATTGCCAGAGAGCCCAGCGCTCC  
 TTCAATCCCTCCTCCAGCCTATCAGTCCTCCCAGCAGCGGGGCATGCAGC

AGCGCCTCCAACTCCAGCCCCAAGAACCATGCCGCCTGCTAAGCCCCAGC  
CTCCAGCCCCGGCCTCCACCTCCTGTGCTTCCTGCAAACCGAGTTCCTCCTG  
CTTCTGCTGCTGCTGCCCCCTGCAGGCGTGGGGACGGCTTCAGCAGCGCCG  
CCACAGACCCCTGGCTCTGCTCCCCCGCCACAGGCTCAGGGACCACCATA  
5 CCCTACCTATCCAGGATATCCCGGGTATTGCCAAATGCCCATGCCCATGGG  
CTACAACCCCTACGCATATGGCCAGTACAATATGCCGTACCCACCGGTGT  
ATCACCAGAGCCCCGGACAGGCTCCATACCCAGGACCCAGCAGCCTACC  
TACCCCTTCCCTCAGCCCCCGCAGCAGTCCTACTATCCACAGCAGTAA

10 Mouse YNK1 partial protein - Sequence ID NO:16

LRAEGAKFRAVLDKAVQADGQVKERYQSHRDTIALLLCKPEPELNAAIPSANP  
AKTMQGSEVVSVLKSLLSNLDEIKKERESLENDLKSVMFDMTSKFLTALAQD  
GVINEEALSVTELDRIYGGTTSKVQESLKKQEGLLKNIQVSHQEFSSMKQSN  
EANLREEVLKLNLAAYDNFVELVANLKEGTFYNELTEILVRFQNKCSDIVFA  
15 RKTERDELLKDLQQSIAREPSAPSIPPPAYQSSPAAGHAAAPPTPAPRTMPPAK  
PQPPARPPPPVLPANRVPPASAAAAPAGVGTASAAPPQTPGSAPPPQAQGP  
PTYPGYPGYCQMPMPMGYNPYAYGQYNMPYPPVYHQSPGQAPYPGPQQPT  
YFPQPPQQSYYPQQ

20 Mouse novel cDNA clone 4: - Sequence ID NO:17

GGTCTTGGCTAGAATTTTAAATTTCTTCTCATTTGAGTAAAATGTTGCATTC  
TGAAGTCCCATGCTACCTGAAGTTGCATTTGGAGTCCCAAGCTACTGGAAT  
GTTTATATGTGACCGTTTCCCAGGAGGCTTACACTGCAGAAGGAAGAATG  
AATCTAGGTGAGGTGGGCAGCTGCTTGGCAGTCCTCTCTTGTGCCCCAACT  
25 GTAAACCAGATAGAAATGTTTCAGGGGAGGATACTTTTATTATTGTGGTTTG  
TAGTGTTAAGATGATTGCTTCTGCCTTGGAAATACCTCAAGCTGTTCTTAT  
TTAACAGGTAAGTGACTGAGTATAATATCCAGAAAAATTTGAAATCCTA  
ATTTCTTCCATATTTTCAATTAATTTTTTGCATACAGGTCTAACAAATATGGA  
TATGTATACACATCCTCTTTAATGAAGGTATTATTTTGGTTACTTTTCCTAA  
30 GATATACCTTAAAAGATGTTCTATACATTTCTTAAATTCTGGGGGAT  
TTGGAGTATGTACATGATAAAAAAGATTATAATATATCGATTGAAGTTACT  
TTATTTTCTAATTAGAATTATTTTAATAGTCCTTTATTGAATAAGTGCTGTA  
ATTTGTTTGCTATGAGACTTATTCCTGATGTGAATGTAAATTATTTTCCAC  
ATGCATGAAAAAATGTATGTACTAATCAGAGTTGTCTCCATTGCATTGAAA  
35 TTAAGTTGTTTGAAGTAAAGTAACTCATATTTATGTAGTAGAATGCTTATG  
TTTTCAGACTTTGTAATGATTTTCTTTGGATGTATTTTAAATCAATCGGTCT  
GGGTAACATATCAGTTTAGATTAATATGTGCTTAAAAGAAGAAAAAATT

- CAATGGTTCATAGTAGAAATGTGCCACACTTAAATAAGCTCTGTATGACAT  
 GAAATTCTGTGTTAAACATTGTAATTCATGGTGACTTTTAACTTATAAAAAT  
 ACTACTTGACGGGTACTTGATTTATGGATATATGAAAACCTCTCAGGAC  
 GAAAGTTCTTCTTTCTCTAGAACTATTCTTCTGTCTGGTCATGCAGAATGCT  
 5 GTTATTCTGAAAAGTGTCCCTGTTGCATATGATGGTCACTTTATTTGGGGG  
 GATTCTTCATAAGATGTGAGATGTTGATGCCAGTCTTTCCCAAGTAAGTGC  
 TCGTAAAAAAGGACTACTAACTAGCCTGCATCTGTCTCTAACTGGGACCA  
 AGGGGTCTGCTGAAGGAACTGAAGAGCTCTAACATTTTCACAGCTTGGA  
 GAAGATAGAATCTTTAAAGTACAACCTGAAGCTTGATCTATTTTACAAGT  
 10 GCATTGATGGCCCCTGTCCTTCTCTGGTTCCTGTCATTTGAAACCAACTCCT  
 GTTGTAATAGGAAGAATATGGGACATTCATATTTAAGAAAATTTGATGT  
 CATTAGGTGACTAAGTAGAAGGCTTAGAAAAATGTATTCATTTGCAAGTA  
 TTTTGGCACAAGAAATTTTCCAACCTGAATAGTAAGCAAAAGCTAAGTTGTT  
 TCATTGAAATCATAAGGCAGTTTAAGATAAACTGGAGAAGATAACTGTTC  
 15 TAATAGAGGATAATCGAATTGATTGTCAAGTGGATGTTATTTATTGGATAG  
 TGACAGAGTTTATTTGTAACCTTAATTATATTAATAAGTTATTCTGTAGGA  
 TGTTTTGTATTAATAAACGTGAACAAAATTAACAAAAAAAAAAAAAAAAAAC  
 TCGAGGG
- 20 Mouse Novel cDNA clone 8/47/52: - Sequence ID NO:18  
 GAGAAGGCGGCCTGCCGCAGCGGGACAACCTAGAGCGCGACGTGGAGGC  
 GCGTAGCGGAGCTGGAGCAACTGCGCACCGAGGTGGATGTGCGCATTAGC  
 GCNNTGGACACCTGCGTCAAGGCCAAGTCGCTGCCAGCCGTCCCGCCGAG  
 AGTCTCAGGCCCACCCCGAACCCTCCACCCATTGATCCAGCTAGCCTGG  
 25 AGGAATTCAAGAAAAGGATCCTGGAGTCTCAGCGGCTCCCTGTAGTCAAC  
 CCTGCWGCCCAACCCAGCGGTTGAGRACCCAGCTGCCGCAGGACGCTGG  
 GTGCCAGAATCGCCACCTGTGGATGGGGGCAGCCAGGTGCCACAGTGC  
 TGGACACCCGCCGTGCCTGCCGGCAGCCTCCACCCCAAGCGCCTTCTCTGG  
 CACCCCTTCACTGTCCCSTGCATCCCCRCCATTCCSSCASWSASKGGATTAA  
 30 GGCACACACAGCTGTGAGATGACTTCACATCGACCCCTTGTGCAGTGACC  
 CGGATGGTGCCCCACCCACACATGAAGCACCCACAGCTCAGCTGCCACCC  
 TAGGCAACTCCTCCGGTTTCCTATCACTCTGCTCCTGACCCGGGAGGTGAG  
 AACAGGAAGCCCAGCCTTCAGCTCCCTTGGGAGTTTCCAGCCTCCCTCTTA  
 AAGGCCACTAGGGTTTCCAGATCCTATTTGAGAGTCTCCAGGCCTCCCTTG  
 35 AAGGGTTCTAGCCACCACGCCCACAGGATTCCCATTAGGTTTTAAAGTCTT  
 TTCCAGAGTCCGCTGGTTCCCCTCCTCCTACAAGGAAGGGCCTCAATTGT  
 AGATGAGCGTTCCGGGTGGATCTTAGAGCCCTAGAGGGAGGCTTTTGCTT

-55-

GTARCCCCCTAAAGATATTACTGGCACATAATAAATATGAAAGTCCTTTG  
AAA  
GTTGGACACTGCGCAAATGGGGCTCTCCATGGACCGCAGCCCATACGCCC  
GCACGGGGGACCAGCAGCGCGGCTCTGGTTCTACCTGCGCTATTTCTTCCT  
5 CTTCGTGTCGCTCATTCAAGTTCCTCATCATCCTGGGCCTGGTCCTCTTCATG  
ATCTATGGCAATGTGCACGCCACCACTGAGTCCAGCCTGCGCGCCACGGA  
GATCCGCGCCGACAGCCTGTACAGCCAGGTGGTTGGACTATCGGCCTCAC  
AGGCTAACCTGAGCAAACAGCTGAACATCAGCTTGCTTGTCAAGGAAACA  
GTCATGCAGCAACTGTTGACTACGCGACGTGAGATGGAGCGCATCAACGC  
10 CAGCTTCCGCCAGTGCCAAGGCGACCTGATCACCTACATAAACTATAATC  
GCTTCATCGCCGCTATCATCCTGAGCGAGAAGCAGTGCCAGGAACAGCTG  
AAGGAGGTCAACAAGACCTGCGAACTTTACTCTTCAAGCTGGGAGAAAAA  
GTTAAGACACTGGAGATGGAGGTGGCCAAGGAGAAGGCAGTGTGCTCCA  
AGGACAAGGAGAGCCTGCTGGCAGGAAAGCGGCAGACGGAAGAGCAGCT  
15 GGAGGCCTGTG

Mouse Novel cDNA clone 18/25: - Sequence ID NO:19

5'end of partial clone

TGTGCGCCGCCTCTAGAACTAGTGGATCCCCCGGGCCTGCAGGAATTCCG  
20 GCACGACGGCCGAGCGCCGCGGACCACCCGCGGCTGCCCCGCCGAGCCGTC  
GACATGTGGGGGGACTGGGGTGGGAGCGGCCGGAGCAGCGCCAGGTACC  
CGGGCGCGCAGAACCATGGCTCTCGCTCGCCTGTCCTGACCTGGCTTGCTC  
GCCCCACCGAAGAATGTCAGCCAAGTCCAAGGGGAACCCTCCTCGTCCTC  
CGCAGCCGAGGGACCGCCGGCAGCCTCCAAAACCAAGGTGAAGGAGCAG  
25 ATCAAGATCATAGTGGAGGATCTGGAATTAGTCCTGGGCGACCTGAAGGA  
CGTGGCCAAAGAACTTAAGGAGGTGGTTGACCAGATTGACACCCTGACCT  
CTGATCTACAGCTGGAAGATGAGATGACCGACAGCTCCAAAACAGACACT  
CTGAACAGCAGCTCCAGTGGGACAACAGCCTCCAGCATAGAGAAGATCA  
AAGAACAGGCCAATGCTCCCCTCATTAACCTCCAGCACACCCGTCTGCT  
30 ATCCTGACTGTCCTGAGAAAGCCAAACCCTCCACCGCCTCCTCCAAGGTTG  
ACACCCGTGAGGTGTGAAGAGCCTCAGAGAGTGGTGCCGACTGCCAACCC  
TGTAAGACCAATGGCACTCTTCTGCGGAATGGAGGCTTAGCGGGGAGGC  
CCAACAAAATTCCAAATGGAG

35 3' end - Sequence ID NO:20

CTCGAGTTTTTTTTTTTTTTTTTTTTTTTTTTTCATTATTTACTATTATTTATT  
GACATATTTCCAAAGCTCAAAATATTTTATTATACATATAGTTGAACATAT

5 GTTTCAAATTGTATAGTATAGAAAATAAACTTTTTTGTAGTGTCTCAGCA  
TTTCATGATGCAAACTATTGACAAACATCTTTAGAAAAATAATAAAATA  
GTCCTTCGGTATTAATAATTCTTATTAATAAGCATTAGATCAAAGGGAGAA  
CTATGACATCATCAATGCATAGATGAGATAGGCATGAATGGAATGAGTTG  
10 CCCTGGCTTTATCAACAAATCAAAATATCTGACATCCCAGCTCTTATAATA  
GACCAAAATACTTGGGAATCAGAAGGTCACAGTTTGTTTTAGGTCAATCAC  
AAAAAAATAAAATTCATTCATACTTTCTCAATTTTCCGCAGTTTCTGATGA  
TGGAACATAGAAAACAATGTACGTCCAGGACAGAGGGCGCTACTCTGCATA  
CTTACCACGTGATTTTTTATGCCACTTTGTTGAATGCAGATTAATATATTTG  
15 GGCTTTTTATTGCTTGAGTAGAAAGTGCTCATTACTTATTATTTTACGTTTA  
TCATATAGAAAATTAATAACAAACAGAACGTTTTCTTAAATGGCAGATAT  
CACACTGTGGTAGTGGTGGATTTCCTCAGGATGGTCTTCTGTGGTTTTGGT  
GCAGCGGGAGGAGGCACGGTTGCAGGTGTGGGAGGGGGGAACTGTTAC  
TGTGGCTTATTCCCAGTCCCCCATTTTCTAATGGGAAAT

15

Mouse Novel cDNA clone 95/ EST accession #AA119951: - Sequence ID NO:21

GCACAGCCCCCTCCATCCTGAAGAAAACCTCAGCGTATGGGCCTCCAGC  
TTCGGGGCCGTGTCTATCCTTCCTCTCCTGGGACATGGTGTTCCTCCGCTTGCC  
CCCCTGGCAGAAAACCG

20

Mouse Ese1L cDNA: Sequence ID NO:22

CGGCACGAGGAGGAGTGGAGCGGCGCGGGGAGGGCGCGCAGCTTGTTG  
CTCCGTAGTACGGCGGCTCGCAAGGGAGCATCCCGAGCGGGCTCCGGGAC  
25 GGCCGGGAGGCAGGCAGGCGGGCGGGCGGGGATGGTGTGCGCGGCTGCG  
GACTCGGCGTTTCCTCGCGCGGCGTGCGGGCTGCACTGATTTGTGTGAGGG  
GCGGCCGCGCGCACCCGCCGAGATGAGGCGTCGATCAGCAAGGTGAA  
CGTAATAGAACCATGGCTCAGTTTCCCACACCTTTCGGTGGTAGCCTGGAT  
GTCTGGGCCATAACTGTGGAGGAAAGGGCCAAGCATGACCAGCAGTTCCT  
30 TAGCCTGAAGCCGATAGCGGGATTTATTACTGGTGATCAAGCGAGGAACT  
TTTTTTTCCAATCTGGGTACCTCAGCCTGTCTTAGCACAAATATGGGCGC  
TAGCGGACATGAATAACGATGGAAGGATGGATCAAGTGAATTTTCCATA  
GCCATGAAGCTTATCAAACCTGAAGCTACAAGGATATCAGCTCCCCTCCAC  
ACTTCCCCCTGTCATGAAACAGCAACCAGTGGCTATTTCCAGTGCACCAGC  
35 ATTTGGTATAGGAGGGATTGCTAGCATGCCACCACTCACAGCTGTTGCTCC  
TGTGCCAATGGGCTCCATTCCAGTTGTTGGAATGTCTCCACCCTTAGTATC  
TTCTGTCCCTCCAGCAGCAGTGCCTCCCCTGGCTAACGGGGCTCCTCCCGT



CATACAGCCTCTGCCTGCGTTTGCGCATCCTGCAGCCACATGGCCAAAGA  
GTTCTTCCTTCAGCAGATCTGGTCCAGGGTCACAATTAACACTAAGTTAC  
AGAAGGCACAATCATTCGATGTGCGCCAGCGCCCCTCCAGCAGCAGAATGG  
GCTGTGCCTCAGTCATCAAGGCTGAAATACAGGCAGTTATTCAACAGCCA  
5 CGACAAAACCTATGAGTGGACACTTAACAGGTCCCCAGGCAAGAACTATTC  
TCATGCAATCAAGTTTACCCCAGGCTCAGCTGGCTTCAATATGGAATCTTT  
CTGACATTGATCAAGATGGAAAACCTACTGCAGAAGAATTTATCCTAGCT  
ATGCACCTAATTGATGTTGCCATGTCTGGTCAGCCACTGCCGCCCGTCCTG  
CCTCCAGAATACATCCCTCCTTCCTTCAGAAGAGTTCGCTCCGGCAGTGGG  
10 ATGTCCGTCATAAGCTCTTCTTCTGTGGATCAGAGGCTGCCTGAGGAGCCG  
TCGTCAGAGGATGAGCAGCAGCCAGAGAAGAACTGCCTGTGACATTTGA  
AGATAAGAAGCGGGAGAACTTCGAGCGAGGCAGTGTGGAGCTGGAGAAG  
CGCCGGCAAGCGCTCTTGGAGCAGCAGCGCAAAGAGCAGGAGCGGTTGG  
CTCAGCTGGAGCGCGCCGAGCAGGAGAGGAAAGAGCGGGAGCGCCAGGA  
15 GCAGGAGGCCAAGCGGCAGCTGGAGCTGGAGAAGCAGCTGGAGAAGCAG  
CGGGAGCTGGAGCGGCAGCGAGAGGAGGAGAGGAGGAAGGAGATCGAG  
AGGCGCGAGGCCGCAAAACGGGAACCTGGAAAGGCAGCGACAACCTTGAAT  
GGGAACGGAACCGGAGACAGGAACCTCCTGAATCAGAGGAACAAGGAGCA  
GGAGGGCACCGTGGTCCTGAAGGCAAGGAGGAAGACTCTGGAGTTTGAGT  
20 TAGAAGCTCTGAATGACAAAAAGCATCAGCTAGAAGGAAAACCTTCAGGAT  
ATCAGGTGTCGACTGGCAACCCAGAGGCAAGAAATTGAGAGCACGAACA  
AGTCTAGAGAGCTAAGAATTGCTGAAATCACCCACTTACAGCAGCAGTTG  
CAGGAATCTCAGCAAATGCTTGGGAAGACTTATTCCAGAGAAACAGATACT  
CAGTGACCAGTTAAAACAAGTCCAGCAGAACAGTTTGCATAGAGACTCGC  
25 TTCTTACCCTCAAAAGAGCCTTGGAAGCAAAGGAGCTGGCCCCGGCAGCAG  
CTCCGGGAGCAGCTGGACGAGGTGGAGAGAGAGACCAGGTCAAAGCTGC  
AGGAGATTGATGTTTTCAACAACCAGCTGAAGGAACCTGAGAGAGATACAT  
AGCAAACAGCAACTCCAGAAGCAGAGGTCCCTGGAGGCAGCGCGACTGA  
AGCAGAAAGAGCAGGAGAGGAAGAGCCTGGAGTTAGAGAAGCAAAAGG  
30 AAGACGCTCAGAGACGAGTTCAGGAAAGGGACAAGCAATGGCTGGAGCA  
TGTGCAGCAGGAGGAGCAGCCACGCCCCCGGAAACCCACGAGGAGGAC  
AGACTGAAGAGGGAAGACAGTGTGAGGAAGAAGGAGGCGGAAGAGAGA  
GCCAAGCCGGAAATGCAAGACAAGCAGAGTCGGCTTTTCCATCCGCATCA  
GGAGCCAGCTAAGCTGGCCACCCAGGCACCCTGGTCTACCACAGAGAAAG  
35 GCCCCGCTTACCATTTCTGCACAGGAGAGTGTAAAAGTGGTATATTACCGA  
GCGCTGTACCCCTTTGAATCCAGAAGTCACGATGAGATCACCATCCAGCC  
AGGAGATATAGTCATGGTGGATGAAAGCCAGACTGGAGAGCCAGGATGG

CTTGGAGGAGAGCTGAAAGGGAAGACGGGATGGTTCCCTGCAAACCTATGC  
AGAAAAGATTCCAGAAAATGAGGTTCCCACTCCAGCCAAACCAGTGACCG  
ATCTGACATCTGCCCCCTGCCCCCAAACCTGGCTCTGCGTGAGACCCCTGCTC  
CTTTGCCAGTGACCTCTTCTGAGCCCTCCACAACCCCCCAACAACTGGGCAG  
5 ACTTCAGTTCCACGTGGCCCAGCAGCTCAAACGAGAAGCCAGAAACGGAC  
AACTGGGATACGTGGGCGGCTCAGCCTTCTCTGACCGTACCTAGTGCTGG  
CCAGTTACGGCAGAGATCAGCCTTTACCCCAGCCACAGCCACTGGCTCCT  
CCCCATCTCCCGTCCTGGGCCAGGGTGAAAAGGTGGAAGGGCTACAAGCG  
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Mouse Ese1L coding: Sequence ID NO:23

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Murine Ese1L protein: Sequence ID NO: 24

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20 Mouse Ese2L cDNA: Sequence ID NO:25

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-66-

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Murine E2L coding: Sequence ID NO: 26

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Mouse Ese2L protein: Sequence ID NO:27

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SKGPTTRRLLLHEVPTGEVWVRFDLQLFEQKTLL

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[illegible]



## Ese Proteins

**SUBSTITUTE SHEET (RULE 26)**

TABLE 2 - Cont'd

**SH3A**

Mouse Eas1 protein 536  
 Mouse Eas2 protein 537  
 Xenopus Intersectin 538  
 Drosophila Dap160-1 539

Mouse Eas1 protein 710  
 Mouse Eas2 protein 711  
 Xenopus Intersectin 712  
 Drosophila Dap160-1 713

Mouse Eas1 protein 743  
 Mouse Eas2 protein 744  
 Xenopus Intersectin 745  
 Drosophila Dap160-1 746

Mouse Eas1 protein 770  
 Mouse Eas2 protein 771  
 Xenopus Intersectin 772  
 Drosophila Dap160-1 773

**SH3B**

Mouse Eas1 protein 785  
 Mouse Eas2 protein 786  
 Xenopus Intersectin 787  
 Drosophila Dap160-1 788

Mouse Eas1 protein 801  
 Mouse Eas2 protein 802  
 Xenopus Intersectin 803  
 Drosophila Dap160-1 804

Mouse Eas1 protein 828  
 Mouse Eas2 protein 829  
 Xenopus Intersectin 830  
 Drosophila Dap160-1 831

Mouse Eas1 protein 855  
 Mouse Eas2 protein 856  
 Xenopus Intersectin 857  
 Drosophila Dap160-1 858

**SH3C**

Mouse Eas1 protein 873  
 Mouse Eas2 protein 874  
 Xenopus Intersectin 875  
 Drosophila Dap160-1 876

Mouse Eas1 protein 890  
 Mouse Eas2 protein 891  
 Xenopus Intersectin 892  
 Drosophila Dap160-1 893

Mouse Eas1 protein 917  
 Mouse Eas2 protein 918  
 Xenopus Intersectin 919  
 Drosophila Dap160-1 920

Mouse Eas1 protein 944  
 Mouse Eas2 protein 945  
 Xenopus Intersectin 946  
 Drosophila Dap160-1 947

**SH3D**

Mouse Eas1 protein 1009  
 Mouse Eas2 protein 1010  
 Xenopus Intersectin 1011  
 Drosophila Dap160-1 1012

Mouse Eas1 protein 1026  
 Mouse Eas2 protein 1027  
 Xenopus Intersectin 1028  
 Drosophila Dap160-1 1029

Mouse Eas1 protein 1053  
 Mouse Eas2 protein 1054  
 Xenopus Intersectin 1055  
 Drosophila Dap160-1 1056

Mouse Eas1 protein 1080  
 Mouse Eas2 protein 1081  
 Xenopus Intersectin 1082  
 Drosophila Dap160-1 1083

Mouse Eas1 protein 1107  
 Mouse Eas2 protein 1108  
 Xenopus Intersectin 1109  
 Drosophila Dap160-1 1110

Mouse Eas1 protein 1134  
 Mouse Eas2 protein 1135  
 Xenopus Intersectin 1136  
 Drosophila Dap160-1 1137

Mouse Eas1 protein 1161  
 Mouse Eas2 protein 1162  
 Xenopus Intersectin 1163  
 Drosophila Dap160-1 1164

Mouse Eas1 protein 1188  
 Mouse Eas2 protein 1189  
 Xenopus Intersectin 1190  
 Drosophila Dap160-1 1191

Mouse Eas1 protein 1215  
 Mouse Eas2 protein 1216  
 Xenopus Intersectin 1217  
 Drosophila Dap160-1 1218

Mouse Eas1 protein 1242  
 Mouse Eas2 protein 1243  
 Xenopus Intersectin 1244  
 Drosophila Dap160-1 1245

Mouse Eas1 protein 1269  
 Mouse Eas2 protein 1270  
 Xenopus Intersectin 1271  
 Drosophila Dap160-1 1272

Mouse Eas1 protein 1296  
 Mouse Eas2 protein 1297  
 Xenopus Intersectin 1298  
 Drosophila Dap160-1 1299

Mouse Eas1 protein 1323  
 Mouse Eas2 protein 1324  
 Xenopus Intersectin 1325  
 Drosophila Dap160-1 1326

Mouse Eas1 protein 1350  
 Mouse Eas2 protein 1351  
 Xenopus Intersectin 1352  
 Drosophila Dap160-1 1353

Mouse Eas1 protein 1377  
 Mouse Eas2 protein 1378  
 Xenopus Intersectin 1379  
 Drosophila Dap160-1 1380

Mouse Eas1 protein 1404  
 Mouse Eas2 protein 1405  
 Xenopus Intersectin 1406  
 Drosophila Dap160-1 1407

Mouse Eas1 protein 1431  
 Mouse Eas2 protein 1432  
 Xenopus Intersectin 1433  
 Drosophila Dap160-1 1434

Mouse Eas1 protein 1458  
 Mouse Eas2 protein 1459  
 Xenopus Intersectin 1460  
 Drosophila Dap160-1 1461

Mouse Eas1 protein 1485  
 Mouse Eas2 protein 1486  
 Xenopus Intersectin 1487  
 Drosophila Dap160-1 1488

Mouse Eas1 protein 1512  
 Mouse Eas2 protein 1513  
 Xenopus Intersectin 1514  
 Drosophila Dap160-1 1515

Mouse Eas1 protein 1539  
 Mouse Eas2 protein 1540  
 Xenopus Intersectin 1541  
 Drosophila Dap160-1 1542

Mouse Eas1 protein 1566  
 Mouse Eas2 protein 1567  
 Xenopus Intersectin 1568  
 Drosophila Dap160-1 1569

Mouse Eas1 protein 1593  
 Mouse Eas2 protein 1594  
 Xenopus Intersectin 1595  
 Drosophila Dap160-1 1596

Mouse Eas1 protein 1620  
 Mouse Eas2 protein 1621  
 Xenopus Intersectin 1622  
 Drosophila Dap160-1 1623

Mouse Eas1 protein 1647  
 Mouse Eas2 protein 1648  
 Xenopus Intersectin 1649  
 Drosophila Dap160-1 1650

Mouse Eas1 protein 1674  
 Mouse Eas2 protein 1675  
 Xenopus Intersectin 1676  
 Drosophila Dap160-1 1677

Mouse Eas1 protein 1701  
 Mouse Eas2 protein 1702  
 Xenopus Intersectin 1703  
 Drosophila Dap160-1 1704

Mouse Eas1 protein 1728  
 Mouse Eas2 protein 1729  
 Xenopus Intersectin 1730  
 Drosophila Dap160-1 1731

Mouse Eas1 protein 1755  
 Mouse Eas2 protein 1756  
 Xenopus Intersectin 1757  
 Drosophila Dap160-1 1758

Mouse Eas1 protein 1782  
 Mouse Eas2 protein 1783  
 Xenopus Intersectin 1784  
 Drosophila Dap160-1 1785

Mouse Eas1 protein 1809  
 Mouse Eas2 protein 1810  
 Xenopus Intersectin 1811  
 Drosophila Dap160-1 1812

Mouse Eas1 protein 1836  
 Mouse Eas2 protein 1837  
 Xenopus Intersectin 1838  
 Drosophila Dap160-1 1839

Mouse Eas1 protein 1863  
 Mouse Eas2 protein 1864  
 Xenopus Intersectin 1865  
 Drosophila Dap160-1 1866

Mouse Eas1 protein 1890  
 Mouse Eas2 protein 1891  
 Xenopus Intersectin 1892  
 Drosophila Dap160-1 1893

Mouse Eas1 protein 1917  
 Mouse Eas2 protein 1918  
 Xenopus Intersectin 1919  
 Drosophila Dap160-1 1920

Mouse Eas1 protein 1944  
 Mouse Eas2 protein 1945  
 Xenopus Intersectin 1946  
 Drosophila Dap160-1 1947

Mouse Eas1 protein 1971  
 Mouse Eas2 protein 1972  
 Xenopus Intersectin 1973  
 Drosophila Dap160-1 1974

Mouse Eas1 protein 1998  
 Mouse Eas2 protein 1999  
 Xenopus Intersectin 2000  
 Drosophila Dap160-1 2001

Mouse Eas1 protein 2025  
 Mouse Eas2 protein 2026  
 Xenopus Intersectin 2027  
 Drosophila Dap160-1 2028

Mouse Eas1 protein 2052  
 Mouse Eas2 protein 2053  
 Xenopus Intersectin 2054  
 Drosophila Dap160-1 2055

Mouse Eas1 protein 2079  
 Mouse Eas2 protein 2080  
 Xenopus Intersectin 2081  
 Drosophila Dap160-1 2082

Mouse Eas1 protein 2106  
 Mouse Eas2 protein 2107  
 Xenopus Intersectin 2108  
 Drosophila Dap160-1 2109

Mouse Eas1 protein 2133  
 Mouse Eas2 protein 2134  
 Xenopus Intersectin 2135  
 Drosophila Dap160-1 2136

Mouse Eas1 protein 2160  
 Mouse Eas2 protein 2161  
 Xenopus Intersectin 2162  
 Drosophila Dap160-1 2163

Mouse Eas1 protein 2187  
 Mouse Eas2 protein 2188  
 Xenopus Intersectin 2189  
 Drosophila Dap160-1 2190

Mouse Eas1 protein 2214  
 Mouse Eas2 protein 2215  
 Xenopus Intersectin 2216  
 Drosophila Dap160-1 2217

Mouse Eas1 protein 2241  
 Mouse Eas2 protein 2242  
 Xenopus Intersectin 2243  
 Drosophila Dap160-1 2244

Mouse Eas1 protein 2268  
 Mouse Eas2 protein